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Michael Plewa
University of Illinois at Urbana-Champaign

Nandakishore Rajagopalan
Illinois Sustainable Technology Center

Elizabeth D. Wagner
University of Illinois at Urbana-Champaign

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Michael Plewa
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Nandakishore Rajagopalan
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University of Illinois at Urbana-Champaign

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BOD	Biochemical oxygen demand
CHO	Chinese hamster ovary
DMSO	Dimethylsulfoxide
FBS	Fetal bovine serum
LDH	Lactate dehydrogenase
MWF	Metalworking fluid(s)
PMN	Polymorphonuclear leukocytes (neutrophils)
POTW	Publicly owned treatment works
U.S. EPA	United States Environmental Protection Agency

ABSTRACT

This toxicological research was conducted to aid in the development of a multicriteria decision-making tool to facilitate selection of functional yet greener and safer metalworking fluids (MWF). This research also satisfies a long-felt need of workers to understand the health risks associated with MWF and a desire to minimize such risks. Utilization of such a tool is expected to lead to improved MWF purchasing decisions through (i) data dependent decision making transparency, (ii) specific articulation and acknowledgment of the multiple criteria often involved in such choice, and (iii) built-in flexibility to reflect and incorporate site-specific preferences and constraints. The specific objectives of this project were: (i) identification of the key characteristics of MWFs that contribute to their utility, economics, environmental, and health and safety aspects; (ii) identification and evaluation of appropriate measures for the above characteristics; (iii) choice and evaluation of an appropriate decision making model to analyze and synthesize the above information; and (iv) development of a user-friendly format to present the end-results of such an analysis. We also intended to develop and test a transiently stable emulsion mixture for providing machining functionality, ease of maintenance, and a superior safety profile. Toxicity data collected on MWF components collected both through a literature search and through testing can guide the choice of components for developing the transiently stable emulsion.

Twelve MWFs and 10 MWF components were evaluated for their chronic cytotoxicity using an in vitro CHO cell bioassay. For both the MWFs and the MWF components, the range of cytotoxicity was within standard toxic agents regulated by the U.S. Environmental Protection Agency. For the MWF classes there was an overall descending cytotoxicity rank order of MWFs of semi-synthetic, followed by soluble oil, followed by synthetic. For these MWF classes, the CHO cell cytotoxicity assay was highly correlated with *in vivo* pulmonary toxicity measurements in animal models. Our results indicate that for both the MWF and MWF components, a wide diversity of cytotoxicity is present. Our data may be used to select MWFs or MWF components that meet industry requirements and pose the lowest level of toxic hazard.

- The MWF rank order, from most cytotoxic to least cytotoxic, was Castrol 6510 > Castrol Clearedge 6536 > Alusol AU39 > Cimperial 1070 > Vita Edge > Castrol 6519 > TrimSol > TrimE 206nd > Eaton Hocut 763 > Hangsterfer's S506 > Syntilo 9904 > IRMCO Cutting Fluid Product A.

- The MWF component rank order, from most cytotoxic to least cytotoxic, was Atramide 202 > Alkaterg T-IV > Busan 77 > Mayfree 133 > Dover Maylube 112 > AMP95 > UCON EMPL-48 >Mayfree Sulperm HO > UCON 50-HB-6, Dover Klorfree 100.

INTRODUCTION

We have developed a multicriteria decision-making tool to facilitate selection of functional yet greener metalworking fluids by reducing the environmental and health and safety footprint of metalworking fluids (MWFs). This research satisfies a long-felt need of workers to understand the health risks associated with MWF, and a desire to minimize such risks by appropriate choices. Utilization of such a tool is expected to lead to (i) improved MWF purchasing decisions through data dependent decision making transparency, (ii) through specific articulation and acknowledgment of the multiple criteria often involved in such a choice, and (iii) built-in flexibility to reflect and incorporate site-specific preferences and constraints. The specific objectives of this project were: (i) identification of the key characteristics of the MWF that contribute to its utility, economics, and environmental and health and safety aspects, (ii) identification and evaluation of appropriate measures for the above characteristics, (iii) choice and evaluation of an appropriate decision making model to analyze and synthesize the above information, and, (iv) development of a user friendly format to present the end-results of such an analysis.

In addition to the above objectives, the project intended to develop and test a transiently stable emulsion mixture for providing machining functionality, ease of maintenance, and a superior safety profile. Toxicity data collected on MWF components collected both through a literature search and through testing will guide the choice of components for developing the transiently stable emulsion.

Background

The higher machining rates and productivity gains achieved throughout the machine-tool industry during the past few decades have resulted in large part from the improved formulation and application of metalworking fluids (MWF). The trend towards higher velocity machining will place even greater demands on MWF [1]. However, the use of MWF is a double-edged sword and brings with it a host of problems. Three key issues associated with the use and disposal of metalworking fluids are: (i) economic, (ii) environmental, and (iii) health and safety.

Economic Issues

MWF are reported to contribute as much as 8 to 16% of machining costs [2], including the cost of the MWF, maintenance, energy use, disposal, and equipment related costs. Many of these costs can be avoided by improving process efficiency, developing and instituting appropriate monitoring and control strategies, and holistic system design. The economics of MWF disposal reveal that the value of raw materials lost in the waste due to disposal can vary from $200 to $400 per 1,000 gallons.

Environmental Concerns

It is estimated that the total volume of MWF being disposed annually is at least 2 billion gallons in the U.S., spread across thousands of small facilities. MWF are typically high in oil content, surfactants, chelates, and biocides [3]. The resulting effluent stream imposes a significant stress on the nation's water treatment plants and waterways. Typical disposal is often into POTW where they create problems such as high BOD loading. The MWF additives create additional wastewater treatment problems such as lowering the efficacy of heavy metals precipitation and increasing emulsification of oil and foaming [4]. The quality of effluent streams produced by widely used waste treatment options such as chemical treatment or ultrafiltration vary widely [5, 6].

Health and Safety Impacts

Significant concerns exist as to the adverse health effects on machinists and workers associated with MWF. The primary adverse health effects due to MWF exposure are (i) bacterial-mediated respiratory disease, (ii) dermal and systematic toxicity, and (iii) the induction of cancer. The isolation of pathogenic or potentially pathogenic organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Legionella* from MWF raise issues of worker safety. One notable example of disease related to MWF is an outbreak of Pontiac fever in an engine manufacturing plant due to *Legionella* [7]. It has also been established quite clearly that large quantities of antigenic bacterial components are present in MWF aerosols [8, 9]. One important example of antigenic material in MWF is endotoxin, a large molecular weight lipo-polysaccharide-protein complex that is released on microbial lysis or microbial cell wall destruction, usually a mode of action of the biocide additives. Several studies have established direct correlations between respiratory illnesses and endotoxin exposure [10-12]. MWF-mediated dermal and systematic toxicity is a serious and constant issue in occupational health [13-15]. Several studies have associated MWF occupational exposure and the induction of various cancers including cancer of the larynx, rectum, pancreas, skin, scrotum, and bladder [9, 16-18]. Recent studies have focused attention on the induction of MWF-associated cancer of the colon-rectum [19], prostrate [16] and breast [20]. However, until the present study, no systematic analysis of the toxicity of MWFs or MWF components has been reported.

METHODS

For this project we conducted a mammalian cell chronic cytotoxicity analysis of MWFs or MWF components presented in Table 1.

Table 1. General characteristics of metalworking fluids and metalworking fluid components analyzed in this study.

Metalworking Fluid	Type	Common Use
Castrol Clearedge 6510	Semi-Synthetic	Ferrous Metals
Castrol Clearedge 6519	Semi-Synthetic	Ferrous or Non-Ferrous Metals
Cimperial-1070	Soluble Oil	Ferrous or Non-Ferrous Metals
Eaton Hocut 763	Synthetic	Steel
Hangsterfer's S-506	Soluble Oil	Aluminum, Stainless Steel, Titanium
Syntilo 9904	Synthetic	Ferrous Metals
TrimE 20bnd	Soluble Oil	Ferrous or Non-Ferrous Metals
Trim Sol	Soluble Oil	General Purpose, Ferrous or Non-Ferrous Metals
Vita Edge	Soluble Plant Oil	General Purpose
Alusol AU39	Semi-Synthetic	Automotive Aluminum Alloys
Castrol Clearedge 6536	Semi-Synthetic	Grinding Operations
IRMCO Cutting Fluid Product A	Synthetic	General Purpose
Busan 77	–	MWF component
UCON EMPL-48	–	MWF component
AMP95	–	MWF component
UCON 50-HB-6	–	MWF component
Alkaterg T-IV	–	MWF component
Actramide 202	–	MWF component
Dover Mayfree 133	–	MWF component
Dover Sulperm HO	–	MWF component
Dover Maylube 112	–	MWF component
Dover Klorfree 100	–	MWF component

Biological Assays

Maintenance of CHO Cells

Stock cultures of Chinese hamster ovary (CHO) line AS52, clone 11-4-8 cells were frozen in a solution of 90% fetal bovine serum (FBS):10% dimethylsulfoxide (DMSO) (v/v) and stored at −80°C. Cells were grown on glass culture plates in Hams F12 medium plus 5% FBS at 37°C in a humidified atmosphere of 5% CO_2. The cells exhibit normal morphology, express cell contact inhibition and grow as a monolayer without expression of neoplastic foci. CHO cells were transferred when the culture became confluent (Figure 1) [21, 22].

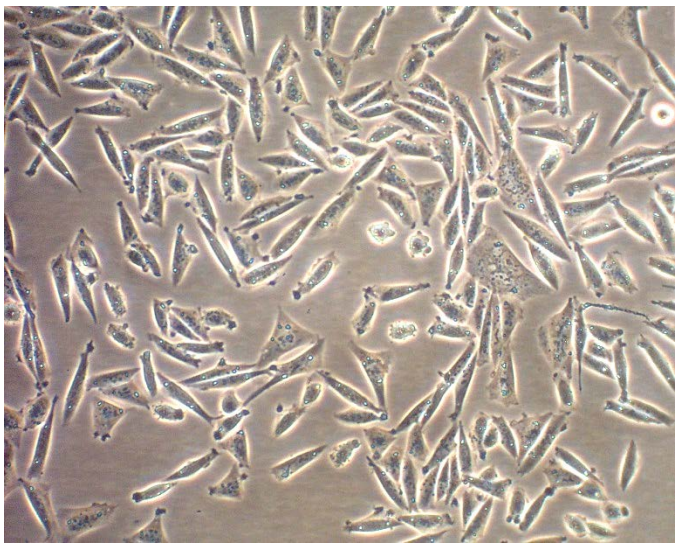

Figure 1. A microphotograph of CHO cells from line AS52, clone 11-4-8. CHO cells are widely used in toxicology research. These cells express functional p53 protein and are competent for DNA repair. The cells exhibit normal morphology, express cell contact inhibition and grow as a monolayer without expression of neoplastic foci.

Solubility of MWF or MWF Components

Using a biological assay demands that the test agents be soluble or be emulsified in an aqueous solution for presentation to the CHO cells. Most of the MWF agents were soluble or formed an emulsion with water; however, several of the MWF components had solubility difficulties. These are the agents that are being incorporated into the on-site combination of MWF components. To dissolve some of these materials, we used a 50% solution of water and dimethylsulfoxide (DMSO) and an ultrasonic probe to disperse the agent into a solution. For the more difficult MWF components, we used a number of emulsifiers and emulsifiers plus DMSO solutions for increasing the solubility of the MWF components. The use of emulsifiers proved to be unacceptable because they dissolved the cell membranes. We finally found that a 1:1 (v/v) mixture of DMSO:ethanol allowed the suspension of the Dover MWF components. The initial Dover MWF components were then diluted serially in F12 medium with FBS for use with the CHO cell chronic cytotoxicity assay. All experiments were conducted at concentrations of the solvent carrier that was below 0.5% which was not toxic to the CHO cells.

CHO Cell Chronic Cytotoxicity Assay

This assay measured the reduction in cell density as based on crystal violet staining as a function of MWF concentration, over a period of approximately 3 cell divisions (72 h) (Figure 2) [23]. This assay has been used to quantitatively compare the levels of mammalian cell cytotoxicity induced by drinking water disinfection by-products [23-32], pesticides [33-36], and medical agents [37], and is being used to study complex mixtures isolated from environmental samples [38]. The data were automatically recorded and transferred to an Excel spreadsheet in a microcomputer connected to the microplate reader. The blank-corrected absorbency value of the negative control (cells with medium only) was set at 100%. The absorbency for each treatment group well was converted into a percentage of the negative control. In general for each MWF sample, approximately 10 concentrations with 8 replicate wells were analyzed per experiment and the experiments were repeated two to three times (Figure 3).

From the summary data, we plotted a cytotoxicity concentration-response curve for each MWF analyzed. The data were curve fitted and regression analysis was conducted. The coefficient of determination (R^2) was determined and the %C½ value for each concentration-response curve was calculated. The %C½ value (analogous to a LC_{50} value) is the concentration of the test agent, determined from a regression analysis of the data that induced a cell density of 50% as compared to the concurrent negative control. The data from the cytotoxicity experiments were transferred to Excel spreadsheets and analyzed using the statistical and graphical functions of SigmaPlot 8.02, SigmaStat 3.1, and Table Curve 4.03. The crystal violet absorbancy data collected by the Bio-Rad microplate reader was saved as a text file (.txt) with the experiment number and transferred to an Excel spreadsheet. The original absorbancy data, the blank-corrected, and the conversion to the percent of the negative control values were saved on the spreadsheet for each MWF analyzed. For each MWF, a summary page was prepared and all of the statistical data was conducted on the percent of the negative control values. A concentration-response cytotoxicity curve for each MWF was generated from the summary page and a one-way analysis of variance (ANOVA) test was conducted to determine if the MWF induced a significant level of cell killing at a specific concentration. If a significant F value of $P \leq 0.05$ was obtained,

a Holm-Sidak multiple comparison versus the control group analysis was conducted. The power of the test statistic was maintained as ≥0.8 at $\alpha = 0.05$.

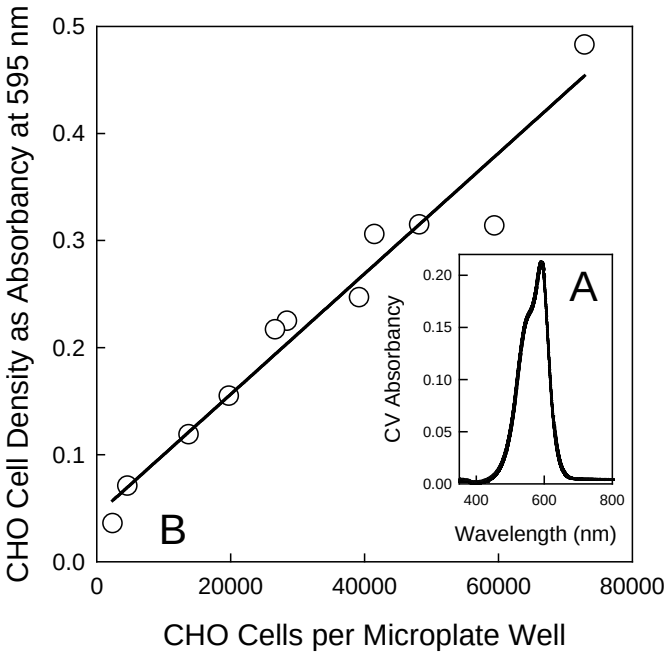

Figure 2. **Calibration of the CHO cell cytotoxicity assay.** (A) Absorption spectrum of crystal violet in the range from 340–800 nm. The maximum absorbancy of crystal violet was between 585–595 nm. (B) A comparison of the number of cells per microplate well determined by Coulter counting or by the absorbancy after crystal violet staining.

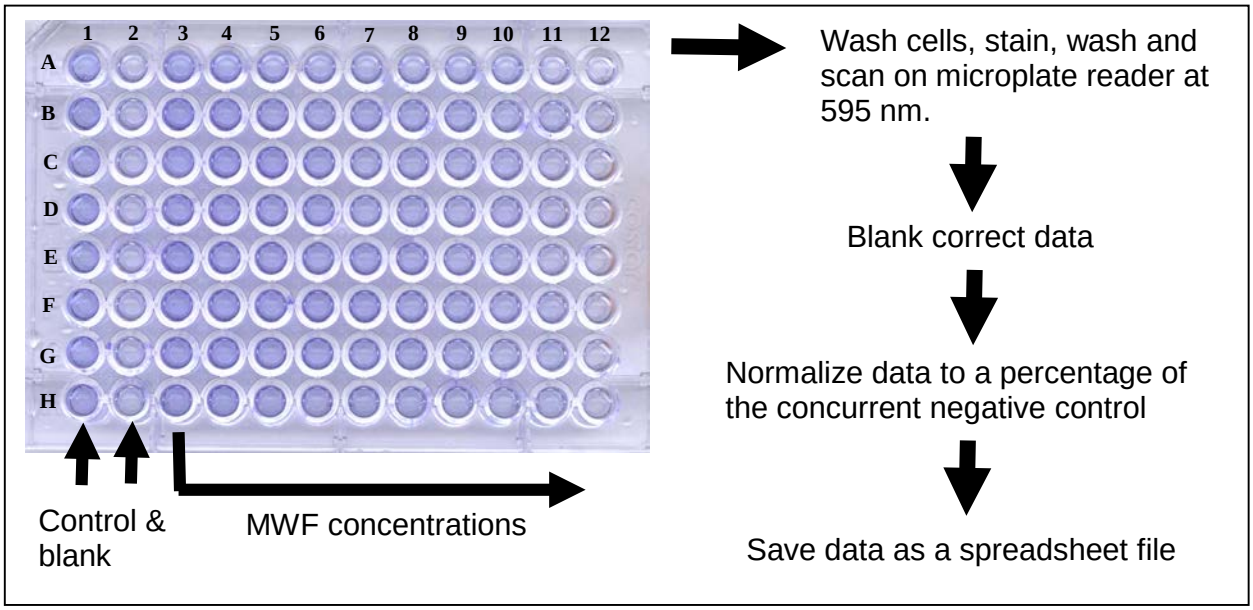

Figure 3. A stained microplate illustrating the CHO cell chronic cytotoxicity assay. The control (column 1) contained cells not exposed to the test MWF agent (negative control). The blank column without cells was used to determine the absorbancy of the crystal violet histological dye that was not associated with cells and to normalize the absorbancy data. The MWF or MWF component was assayed from low concentration (column 3) to high concentrations.

RESULTS

The results of the experiments preformed on the metalworking fluids and MWF components listed in Table 1 are presented in the following tables and figures.

Metalworking Fluids

Castrol Clearedge 6510

The summary data for the CHO cell cytotoxicity assay for Castrol 6510 is presented in Table 2 and the statistical analysis is presented in Table 3. Figure 4 presents the cytotoxicity concentration-response curve for the MWF.

Table 2. **Summary of CHO cell cytotoxicity of Castrol 6510.**
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 031006A 033106A 040406A Castrol 6510 MWF CHO Cytotoxicity % NEGATIVE CONTROL SUMMARY									
0	5	10	15	20	25	30	35	40	
125.69	88.35	97.56	53.66	60.43	98.92	74.53	33.33	-19.24	
103.40	108.67	86.18	77.78	76.96	105.42	66.94	60.98	-28.18	
97.03	116.53	104.07	60.98	120.33	100.81	72.36	60.70	19.24	
102.76	112.47	94.31	102.71	127.37	86.72	56.10	29.00	18.43	
85.56	114.36	106.78	89.16	118.43	117.89	83.47	31.71	21.95	
94.69	121.41	92.14	99.19	94.31	60.16	73.44	28.18	24.93	
103.40	128.18	123.85	131.44	139.02	80.76	59.62	24.39	29.00	
87.90	67.48	98.64	95.66	108.13	37.67	37.67	-22.22	34.96	
52.98	129.47	152.66	88.40	99.37	103.45	39.50		-6.58	
83.70	120.69	98.75	79.62	99.69	88.09	32.60		-3.13	
65.52	113.79	106.27	77.12	71.47	67.08	30.09		-25.39	
105.02	97.81	96.87	104.70	53.61	78.37	57.37		-27.27	
115.67	130.41	103.76	93.73	69.91	74.92	-1.57		-17.55	
99.06	111.29	92.79	101.57	54.23	51.72	7.52		-22.57	
145.14	130.72	105.33	105.64	58.93	66.14	46.08		5.02	
133.54	110.34	131.03	76.18	38.87	34.48	-0.31		-7.84	
105.96	92.36				42.68				
119.51	91.08				36.31				
103.52	89.60				33.76				
99.46	68.37				39.92				
86.99	67.94				31.85				
78.59	71.97				24.20				
117.34	75.37				37.15				
88.08	61.78				29.72				
24.00	24.00	16.00	16.00	16.00	24.00	16.00	8.00	16.00	number
100.02	100.85	105.69	89.85	86.94	63.67	45.96	30.76	-0.27	Average
4.19	4.64	4.23	4.74	7.61	5.93	6.76	9.14	5.55	SE

Table 3. Statistical analysis of the CHO cell cytotoxicity data of Castrol 6510.

Data source: Data 7 in MWF 122706.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
6510 0 µg/mL	24	0	100.021	20.518	4.188
6510 5 µg/mL	24	0	100.852	22.708	4.635
6510 10 µg/mL	16	0	105.686	16.909	4.227
6510 15 µg/mL	16	0	89.846	18.945	4.736
6510 20 µg/mL	16	0	86.942	30.421	7.605
6510 25 µg/mL	24	0	63.675	29.061	5.932
6510 30 µg/mL	16	0	45.963	27.022	6.755
6510 35 µg/mL	8	0	30.759	25.842	9.136
6510 40 µg/mL	16	0	-0.265	22.190	5.547

Source of Variation	DF	SS	MS	F	P
Between Groups	8	174655.959	21831.995	37.661	<0.001
Residual	151	87533.363	579.691		
Total	159	262189.322			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
6510 0 µg/mL vs. 6510 40 µg/mL	100.287	12.906	3.755E-026	0.006	Yes
6510 0 µg/mL vs. 6510 35 µg/mL	69.262	7.047	6.092E-011	0.007	Yes
6510 0 µg/mL vs. 6510 30 µg/mL	54.058	6.957	9.887E-011	0.009	Yes
6510 0 µg/mL vs. 6510 25 µg/mL	36.346	5.229	0.000000558	0.010	Yes
6510 0 µg/mL vs. 6510 20 µg/mL	13.079	1.683	0.0944	0.013	No
6510 0 µg/mL vs. 6510 15 µg/mL	10.176	1.309	0.192	0.017	No
6510 0 µg/mL vs. 6510 10 µg/mL	5.665	0.729	0.467	0.025	No
6510 0 µg/mL vs. 6510 5 µg/mL	0.830	0.119	0.905	0.050	No

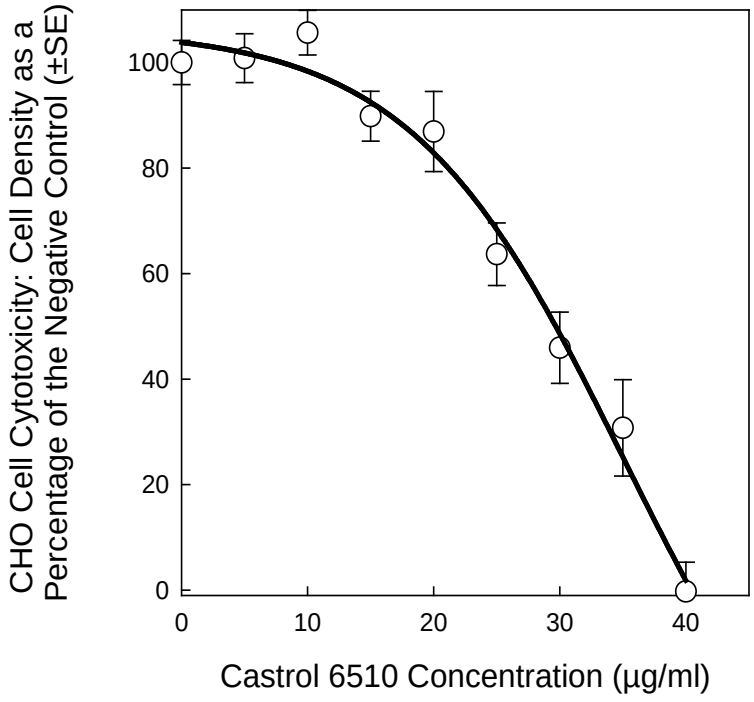

Figure 4. CHO cell chronic cytotoxicity concentration-response curve for Castrol 6510.

Castrol Clearedge 6519

The summary data for the CHO cell cytotoxicity assay for Castrol 6519 is presented in Table 4 and the statistical analysis is presented in Table 5. Figure 5 presents the cytotoxicity concentration-response curve for the MWF.

Table 4. Summary of CHO cell cytotoxicity of Castrol 6519.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiments 011105, 012505, 020105 Castrol 6519 MWF SUMMARY SHEET CHO Cytotoxicity % NEGATIVE CONTROL													
0	2.5	5	12.5	25	37.5	50	60	75	85	100	125	150	µg/ml
0	0.5	1	2.5	5	7.5	10	12	15	17	20	25	30	µg/well
105.56	91.11	76.22	78.44	82.00	86.75	61.56	51.62	61.10	19.24	6.00	13.77	8.61	
91.33	77.11	66.22	68.44	61.33	80.03	39.78	23.92	57.14		10.89	5.85	4.48	
87.33	73.33	70.22	66.00	58.89	71.94	44.44	48.74	65.40	24.82	26.00	12.74	1.89	
91.33	93.78	75.56	70.22	60.67	77.28	43.33	28.06	48.71	28.60	13.78	8.43	3.79	
109.56	86.89	76.89	71.11	62.89	71.94	40.22	50.72	58.86		19.11	15.49	6.54	
93.78	79.56	74.22	66.89	50.67	69.71	40.89	46.22	57.14	16.19	13.78	15.66	2.24	
110.22	88.89	76.67	74.89	64.67	81.07	51.33	70.50	24.10		25.56	13.94	9.12	
110.00	106.00	68.44	82.89	68.67	67.30	44.00		35.07	13.31	22.00	12.22	3.96	
109.12		127.37		97.59	55.58	79.00		23.38		39.76	-7.01	-10.07	
117.21		114.97		78.14	60.07	66.27		34.35		33.22		-3.24	
93.29		103.61		70.91	71.22	69.54		35.79		32.36	8.45	0.00	
89.50		99.66		79.35		63.68		33.99		32.36	-9.35	-5.94	
97.59		104.30		71.26	67.09	65.75				34.94	-8.99	-3.42	
91.57		85.20		79.35	38.85	69.88				32.01	-8.99	-12.05	
110.15		95.70		74.35	44.96	64.37				39.59		-16.19	
91.05		86.75		72.98		66.95				39.76	1.98	10.07	
104.14		101.62		75.36		70.86				10.07			
78.06		90.11		48.20		57.37				20.68			
105.22		62.95		73.74		57.91				21.94			
94.06		71.76		71.58		34.17				2.70			
87.95		71.94		59.35		24.28							
95.68		50.00		44.78		53.42							
111.69						69.60							
123.38						52.34							
24.00	8.00	22.00	8.00	22.00	14.00	24.00	7.00	12.00	5.00	20.00	14.00	16.00	number
99.95	87.08	84.11	72.36	68.49	67.41	55.46	45.68	44.59	20.43	23.82	5.30	-0.01	Average
2.28	3.70	3.99	2.10	2.61	3.63	2.85	5.92	4.35	2.79	2.59	2.64	1.96	SE
11.19	10.47	18.71	5.93	12.22	13.56	13.98	15.66	15.06	6.25	11.58	9.87	7.86	SD

Table 5. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Castrol 6519.

Data source: Data 4 in MWF 073105.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
C6519 0 µg/mL	24	0	99.949	11.188	2.284
C6519 2.5 µg/mL	8	0	87.083	10.470	3.702
C6519 5 µg/ml	22	0	84.108	18.707	3.988
C6519 12.5 µg/mL	8	0	72.361	5.930	2.096
C6519 25 µg/mL	22	0	68.487	12.220	2.605
C6519 37.5 µg/mL	15	1	67.414	13.564	3.625
C6519 50 µg/mL	24	0	55.457	13.976	2.853
C6519 60 µg/mL	7	0	45.683	15.655	5.917
C6519 75 µg/mL	12	0	44.588	15.065	4.349
C6519 85 µg/mL	8	3	20.432	6.247	2.794
C6519 100 µg/mL	20	0	23.825	11.585	2.590
C6519 125 µg/mL	16	2	5.299	9.872	2.638
C6519 150 µg/mL	16	0	-0.0130	7.858	1.964

Source of Variation	DF	SS	MS	F	P
Between Groups	12	194261.377	16188.448	98.330	<0.001
Residual	183	30128.104	164.634		
Total	195	224389.481			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
C6519 0 µg/mL vs. C6519 150 µg/mL	99.962	24.138	8.982E-059	0.004	Yes
C6519 0 µg/mL vs. C6519 125 µg/mL	94.650	21.935	4.135E-053	0.005	Yes
C6519 0 µg/mL vs. C6519 100 µg/mL	76.124	19.595	8.248E-047	0.005	Yes
C6519 0 µg/mL vs. C6519 85 µg/mL	79.517	12.606	1.241E-026	0.006	Yes
C6519 0 µg/mL vs. C6519 75 µg/mL	55.361	12.204	1.906E-025	0.006	Yes
C6519 0 µg/mL vs. C6519 50 µg/mL	44.492	12.012	6.969E-025	0.007	Yes
C6519 0 µg/mL vs. C6519 60 µg/mL	54.265	9.845	1.273E-018	0.009	Yes
C6519 0 µg/mL vs. C6519 25 µg/mL	31.462	8.307	2.137E-014	0.010	Yes
C6519 0 µg/mL vs. C6519 37.5 µg/mL	32.535	7.540	2.114E-012	0.013	Yes
C6519 0 µg/mL vs. C6519 12.5 µg/mL	27.588	5.267	0.000000387	0.017	Yes
C6519 0 µg/mL vs. C6519 5 µg/mL	15.841	4.183	0.0000446	0.025	Yes
C6519 0 µg/mL vs. C6519 2.5 µg/mL	12.866	2.456	0.0150	0.050	Yes

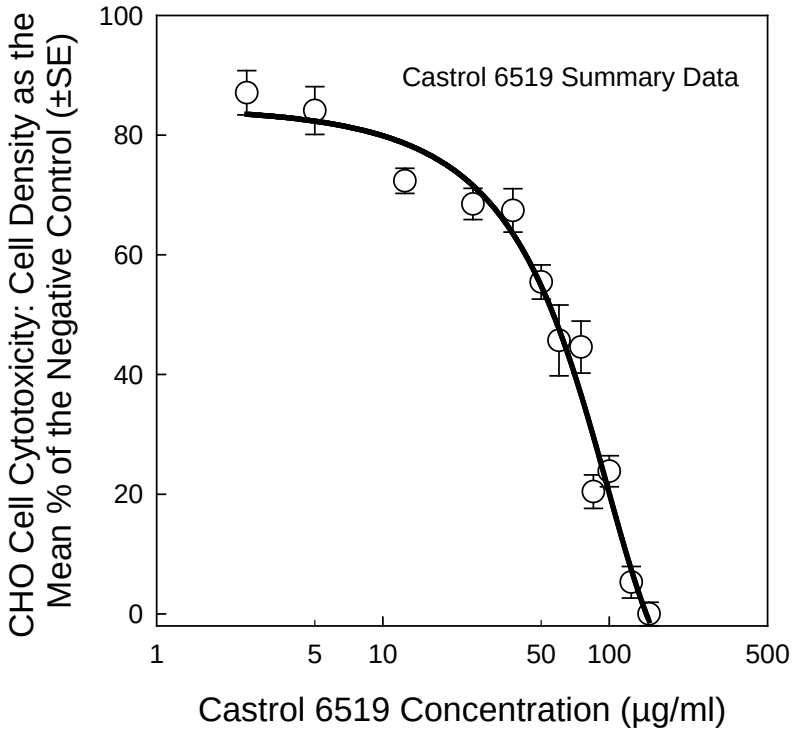

Figure 5. CHO cell chronic cytotoxicity concentration-response curve for Castrol 6519.

Cimperial-1070

The summary data for the cytotoxicity assay for Cimperial-1070 is presented in Table 6 with the statistical analysis presented in Table 7 and the concentration-response curve in Figure 6.

Table 6. **Summary of CHO cell cytotoxicity of Cimperial-1070.**

Experiments 011105JPMP, 030105JPMP, 030805JPMP Cimperial 1070 MWF SUMMARY SHEET CHO Cytotoxicity % NEGATIVE CONTROL																
0	2.5	5	12.5	25	30	35	40	45	50	60	70	75	90	100	125	µg/ml
0	0.5	1	2.5	5	6	7	8	9	10	12	14	15	18	20	25	µg/well
104.4	95.9	93.9	82.1	70.5	84.2	72.1	44.8	48.6	42.1	20.8	1.9	15.9	6.3	13.8	3.8	
95.9	84.1	83.8	82.1	68.1	79.2	66.5	37.7	44.9	35.8	19.8	4.4	22.2	10.5	8.7	-2.5	
88.6	78.2	89.3	87.8	77.3	80.2	74.0	52.7	51.3	33.6	24.7	5.4	23.4	17.2	10.5	-1.7	
93.0	88.2	88.2	77.9	73.1	69.4	69.7	28.0	48.0	32.5	21.8	15.6	17.6	2.5	8.1	-2.5	
101.1	86.5	87.1	84.5	81.2	71.3	62.2	31.0	47.8	40.4	21.0	17.9	17.2	12.1	9.0	-2.9	
103.1	84.9	88.2	91.7	72.9	56.6	57.2	54.0	42.2	28.4	15.6	12.1	8.8	12.1	10.7	-3.8	
114.2	87.6	91.9	91.9	79.7	53.9	46.2	47.7	53.4	39.7	25.8	19.3	22.2	8.8	14.8	0.8	
100.2	97.6	89.1	107.4	102.0	36.2	35.6	56.5	28.5	31.0	11.9	18.7	14.2	6.7	2.6	-1.3	
113.0		92.1	87.0	61.5			68.0		49.8			8.5		4.6		
98.7		90.0	91.2	73.2			63.2		44.8			12.9		4.6		
92.1		94.1	79.5	80.3			58.4		67.4			14.1		9.2		
92.9		85.4	90.8	87.4			52.8		57.7			10.4		3.3		
92.5		102.1	87.9	89.5			40.3		73.2			7.7		7.5		
81.2		92.1	77.0	73.2			59.0		68.2			-0.6		-0.8		
130.5		84.5	82.8	78.7			27.7		54.8			18.5		6.3		
99.2		77.4	69.0	82.0					59.0			-4.4		2.1		
131.8				70.3					30.3					8.3		
102.7				76.5					36.2					6.7		
91.1				85.2					30.8					-2.5		
85.0				71.3					34.5					-3.9		
94.8				67.6					29.9					-4.8		
100.0				61.7					21.8					-5.4		
108.7				39.9					28.9					0.6		
86.3									22.0					-6.2		
24	8	16	16	23	8	8	15	8	24	8	8	16	8	24	8	number
100.0	87.9	89.3	85.7	74.9	66.4	60.5	48.1	45.6	41.4	20.2	11.9	13.0	9.5	4.5	-1.3	Average
2.58	2.23	1.37	2.14	2.48	5.77	4.77	3.30	2.72	3.03	1.61	2.49	1.96	1.59	1.24	0.87	SE
12.63	6.30	5.46	8.57	11.91	16.31	13.48	12.78	7.70	14.83	4.54	7.05	7.82	4.49	6.05	2.45	SD

Table 7. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Cimperial-1070.

Data source: Data 5 in MWF 073105.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
CI1070 0µg/ml	24	0	100.034	12.625	2.577
CI1070 2.5µg/ml	8	0	87.855	6.300	2.227
CI1070 5µg/ml	16	0	89.322	5.461	1.365
CI1070 12.5µg/ml	16	0	85.671	8.569	2.142
CI1070 25µg/ml	23	0	74.925	11.910	2.483
CI1070 30µg/ml	8	0	66.378	16.314	5.768
CI1070 35µg/ml	8	0	60.453	13.478	4.765
CI1070 40µg/ml	15	0	48.109	12.784	3.301
CI1070 45µg/ml	8	0	45.568	7.704	2.724
CI1070 50µg/ml	24	0	41.367	14.830	3.027
CI1070 60µg/ml	8	0	20.183	4.541	1.605
CI1070 70µg/ml	8	0	11.922	7.051	2.493
CI1070 75µg/ml	16	0	13.030	7.822	1.956
CI1070 90µg/ml	8	0	9.519	4.488	1.587
CI1070 100µg/ml	24	0	4.494	6.055	1.236
CI1070 125µg/ml	8	0	-1.255	2.450	0.866

Source of Variation	DF	SS	MS	F	P
Between Groups	15	260054.241	17336.949	162.735	<0.001
Residual	206	21946.173	106.535		
Total	221	282000.414			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
CI1070 0 µg/mL vs. CI1070 100 µg/mL	95.540	32.065	5.020E-082	0.003	Yes
CI1070 0 µg/mL vs. CI1070 75 µg/mL	87.005	26.118	2.733E-067	0.004	Yes
CI1070 0 µg/mL vs. CI1070 125 µg/mL	101.289	24.038	1.082E-061	0.004	Yes
CI1070 0 µg/mL vs. CI1070 90 µg/mL	90.515	21.481	1.731E-054	0.004	Yes
CI1070 0 µg/mL vs. CI1070 70 µg/mL	88.112	20.911	7.804E-053	0.005	Yes
CI1070 0 µg/mL vs. CI1070 50 µg/mL	58.667	19.690	3.077E-049	0.005	Yes
CI1070 0 µg/mL vs. CI1070 60 µg/mL	79.851	18.950	5.020E-047	0.006	Yes
CI1070 0 µg/mL vs. CI1070 40 µg/mL	51.925	15.285	9.368E-036	0.006	Yes
CI1070 0 µg/mL vs. CI1070 45 µg/mL	54.466	12.926	2.238E-028	0.007	Yes
CI1070 0 µg/mL vs. CI1070 35 µg/mL	39.581	9.393	1.132E-017	0.009	Yes
CI1070 0 µg/mL vs. CI1070 25 µg/mL	25.109	8.337	1.078E-014	0.010	Yes
CI1070 0 µg/mL vs. CI1070 30 µg/mL	33.657	7.987	9.607E-014	0.013	Yes
CI1070 0 µg/mL vs. CI1070 12.5 µg/mL	14.364	4.312	0.0000251	0.017	Yes
CI1070 0 µg/mL vs. CI1070 5 µg/mL	10.713	3.216	0.00151	0.025	Yes
CI1070 0 µg/mL vs. CI1070 2.5 µg/mL	12.179	2.890	0.00426	0.050	Yes

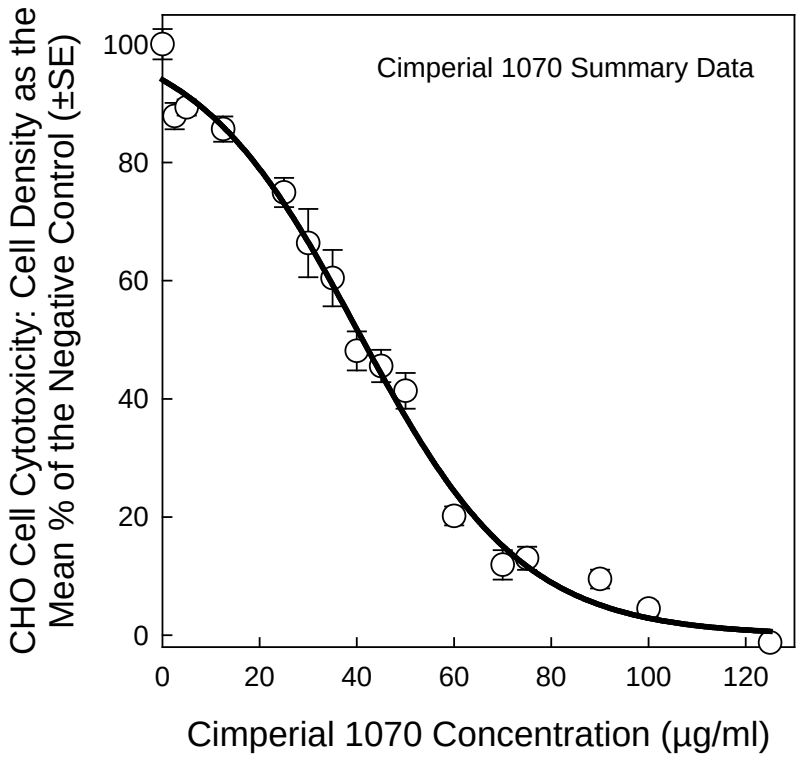

Figure 6. CHO cell chronic cytotoxicity concentration-response curve for Cimperial 1070.

Eaton Hocut 763

The summary data for the CHO cell cytotoxicity assay for Eaton Hocut 763 is presented in Table 8 and the statistical analysis is presented in Table 9. Figure 7 presents the cytotoxicity concentration-response curve for the MWF.

Table 8. **Summary of CHO cell cytotoxicity of Eaton Hocut 763.**
The cell density is a percentage of the concurrent negative control for each microplate.

CHO Cell Chronic Cytotoxicity Data for Eaton Hocut 763									
0	25	50	75	100	125	150	175	200	µg/ml
0	5	10	15	20	25	30	35	40	µg/well
96.76	90.31	97.92	97.58	45.67	46.71	44.98	29.41	38.75	
90.52	99.31	88.24	102.08	67.13	54.67	45.67	26.64	35.99	
101.75	116.26	97.58	95.85	75.78	46.37	39.45	24.91	10.38	
110.22	91.70	83.74	104.84	75.78	58.48	42.91	47.40	22.84	
96.51	120.76	125.61	100.35	66.44	29.41	66.78	16.96	17.99	
111.22	87.54	81.31	101.04	68.51	51.21	16.61	24.22	6.57	
107.48	130.80	80.28	46.02	74.39	75.43	27.34	2.42	10.38	
85.04	74.05	51.56	44.64	44.64	55.36	22.84	37.72	-14.88	
79.24	78.65	76.99	73.34	35.84	24.23	9.73	-7.41	-16.70	
107.61	78.87	69.14	53.65	48.78	28.54	12.83	-2.32	-7.63	
125.95	74.78	55.09	64.93	57.74	25.66	20.13	3.87	-7.63	
82.01	83.30	70.13	46.13	48.23	24.00	16.48	-3.65	-8.63	
129.41	104.31	72.90	49.23	48.12	22.35	17.04	4.98	-10.29	
85.12	95.35	65.38	51.11	51.22	19.14	18.36	1.00	-11.39	
100.69	88.16	72.35	62.06	44.14	16.26	5.09	-0.66	-13.38	
91.00	76.00	74.78	56.42	31.64	22.46	-1.22	-9.40	-16.15	
128.98									
100.66									
95.35									
92.04									
99.56									
92.81									
93.47									
97.46									
24	16	16	16	16	16	16	16	16	Number
100.04	93.13	78.94	71.83	55.25	37.52	25.31	12.26	2.26	Average
2.79	4.31	4.46	6.00	3.58	4.44	4.50	4.35	4.65	SE
13.68	17.23	17.85	23.99	14.32	17.76	17.98	17.41	18.59	SD

Table 9. **Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Eaton Hocut 763.**

Data source: Data 8 in MWF 122706.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
EH763 0 µg/mL	24	0	100.036	13.683	2.793
EH763 25 µg/mL	16	0	93.134	17.235	4.309
EH763 50 µg/mL	16	0	78.936	17.851	4.463
EH763 75 µg/mL	16	0	71.828	23.987	5.997
EH763 100 µg/mL	16	0	55.253	14.325	3.581
EH763 125 µg/mL	16	0	37.517	17.763	4.441
EH763 150 µg/mL	16	0	25.314	17.980	4.495
EH763 175 µg/mL	16	0	12.255	17.408	4.352
EH763 200 µg/mL	16	0	2.263	18.594	4.648

Source of Variation	DF	SS	MS	F	P
Between Groups	8	178339.990	22292.499	71.533	<0.001
Residual	143	44564.233	311.638		
Total	151	222904.223			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
EH763 0 µg/mL vs. EH763 200 µg/mL	97.773	17.160	1.534E-036	0.006	Yes
EH763 0 µg/mL vs. EH763 175 µg/mL	87.781	15.407	3.516E-032	0.007	Yes
EH763 0 µg/mL vs. EH763 150 µg/mL	74.722	13.115	2.692E-026	0.009	Yes
EH763 0 µg/mL vs. EH763 125 µg/mL	62.519	10.973	1.052E-020	0.010	Yes
EH763 0 µg/mL vs. EH763 100 µg/mL	44.783	7.860	8.441E-013	0.013	Yes
EH763 0 µg/mL vs. EH763 75 µg/mL	28.208	4.951	0.00000205	0.017	Yes
EH763 0 µg/mL vs. EH763 50 µg/mL	21.100	3.703	0.000303	0.025	Yes
EH763 0 µg/mL vs. EH763 25 µg/mL	6.902	1.211	0.228	0.050	No

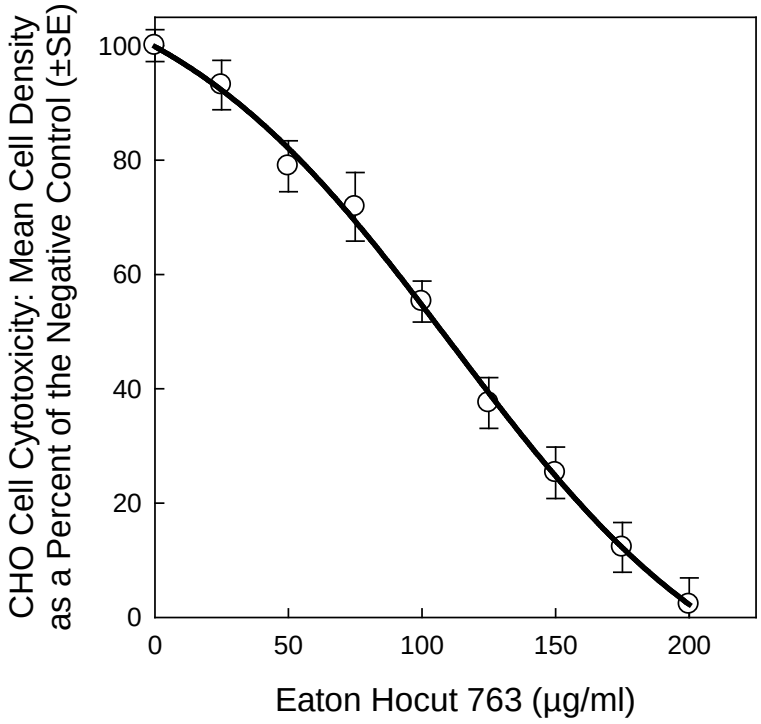

Figure 7. CHO cell chronic cytotoxicity concentration-response curve for Eaton Hocut 763.

Hangsterfer's S-506

The summary data for the CHO cell cytotoxicity assay for Hangsterfer's S-506 is presented in Table 10 and the statistical analysis is presented in Table 11. Figure 8 presents the cytotoxicity concentration-response curve for the MWF.

Table 10. Summary of CHO cell cytotoxicity of Hangsterfer's S-506.

Experiments 0708JPAG, 071105JPAG Hangsterfer's S-506 CF MWF CHO Cytotoxicity % NEGATIVE CONTROL																	
0	5	35	50	60	75	85	100	120	125	140	150	160	200	225	250	300	µg/ml
0	1	7	10	12	15	17	20	24	25	28	30	32	40	45	50	60	µg/well
102.53	94.63	94.34	70.79	53.50	53.50	50.37	49.63		39.34		27.72				-3.43		
103.87	81.97	79.73	73.47	71.68	61.85	53.06	51.71		34.58		25.34				1.64		
122.95	95.38	69.30	71.09	62.00	56.04	43.82	44.56		35.62		31.74				2.09		
92.10	90.01	65.72	76.30	62.30	48.73	50.82	52.31		40.54		30.70				0.15		
94.63	85.84	67.51	71.39	63.93	49.33	49.78	49.78		36.21		26.23				-4.62		
101.64	80.48	69.15	68.26	63.64	54.10	52.31	48.73		33.83		22.50				-5.37		
95.23	86.44	90.01	86.44	66.02	65.13	63.04	47.54		42.92		26.68				-2.09		
86.89	89.72	81.22	78.69	46.94	57.08	53.65	37.11		33.68		19.23				6.11		
93.70			71.85		70.59		69.12	47.69		41.60		34.24	17.23	8.19	-0.84	-1.68	
79.41			47.48		55.25		36.13	40.55		35.71		30.67	-0.21	21.22	0.00	-9.66	
94.54			62.39		63.66		61.34	50.84		40.97		36.34	25.63	28.15	5.04	-5.46	
111.76			71.85		74.79		68.49	56.72		42.65		43.07	23.74	21.43	5.04	-4.62	
122.48			85.50		78.57		69.75	54.20		55.04		40.13	28.36	21.64	15.55	-5.25	
111.55			79.83		81.09		58.82	49.79		44.96		30.67	22.48	15.76	14.08	-6.51	
113.87			76.89		69.96		75.42	59.87		51.47		43.28	24.79	13.87	18.91	-1.47	
			63.03		56.09		55.88	45.59		35.92		32.14	20.80	15.76	11.76	-3.99	
101.09			77.72				86.96	76.09		65.76		70.65	20.65	7.07	22.28	10.33	
76.63			68.48				75.54	75.00		65.22		76.63	32.61	4.89	39.67	-3.26	
94.02			73.91				97.83	97.83		79.35			52.17	34.78	35.33	3.80	
93.48			88.04				92.39	75.00		64.13		63.04	31.52	26.63	19.57	1.09	
90.76			109.24				91.85	95.11		92.93		86.96	36.41	48.91	19.57	13.04	
100.00			117.93				103.26	98.91		71.20		75.54	58.15	33.70	28.80	10.33	
98.91			84.24				116.85	90.76		105.43		93.48	79.89	44.57	28.26		
			83.15					59.78		87.50		35.87	68.48	11.96	17.93	4.35	
22	8	8	23	8	16	8	23	15	8	15	8	14	15	15	23	14	number
99.18	88.06	77.12	77.17	61.25	62.23	52.11	67.00	67.60	37.09	59.49	26.27	54.06	31.62	23.10	11.20	-0.24	Average
2.55	1.92	3.86	3.04	2.71	2.55	1.90	4.64	5.32	1.21	5.44	1.44	6.06	4.97	3.40	2.79	1.88	SE
11.95	5.42	10.91	14.57	7.66	10.21	5.37	22.26	20.59	3.43	21.07	4.08	22.66	19.23	13.16	13.36	7.04	SD

Table 11. **Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Hangsterfer's S-506.**

Data source: Data 3 in MWF 073105.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
HS 0 µg/mL	22	0	99.184	11.947	2.547
HS 5 µg/mL	8	0	88.059	5.419	1.916
HS 35 µg/mL	8	0	77.124	10.910	3.857
HS 50 µg/mL	24	0	77.415	14.304	2.920
HS 60 µg/mL	8	0	61.252	7.661	2.709
HS 75 µg/mL	16	0	62.235	10.207	2.552
HS 85 µg/mL	8	0	52.105	5.368	1.898
HS 100 µg/mL	23	0	67.000	22.260	4.641
HS 120 µg/mL	16	0	67.108	19.991	4.998
HS 125 µg/mL	8	0	37.090	3.432	1.213
HS 140 µg/mL	16	0	61.240	21.530	5.382
HS 150 µg/mL	8	0	26.267	4.080	1.443
HS 160 µg/mL	15	0	52.848	22.335	5.767
HS 200 µg/mL	16	0	33.919	20.738	5.185
HS 225 µg/mL	16	0	22.407	13.020	3.255
HS 250 µg/mL	24	0	11.477	13.140	2.682
HS 300 µg/mL	15	0	0.0679	6.887	1.778

Source of Variation	DF	SS	MS	F	P
Between Groups	16	198361.962	12397.623	52.199	<0.001
Residual	234	55576.962	237.508		
Total	250	253938.924			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
HS 0 µg/mL vs. HS 250 µg/mL	87.708	19.281	0.000	0.003	Yes
HS 0 µg/mL vs. HS 300 µg/mL	99.116	19.207	0.000	0.003	Yes
HS 0 µg/mL vs. HS 225 µg/mL	76.777	15.163	0.000	0.004	Yes
HS 0 µg/mL vs. HS 200 µg/mL	65.265	12.889	0.000	0.004	Yes
HS 0 µg/mL vs. HS 150 µg/mL	72.917	11.460	0.000	0.004	Yes
HS 0 µg/mL vs. HS 125 µg/mL	62.094	9.759	0.000	0.005	Yes
HS 0 µg/mL vs. HS 160 µg/mL	46.336	8.979	0.000	0.005	Yes
HS 0 µg/mL vs. HS 140 µg/mL	37.944	7.494	0.000	0.006	Yes
HS 0 µg/mL vs. HS 85 µg/mL	47.079	7.399	0.000	0.006	Yes
HS 0 µg/mL vs. HS 75 µg/mL	36.950	7.297	0.000	0.007	Yes
HS 0 µg/mL vs. HS 100 µg/mL	32.184	7.003	0.000	0.009	Yes
HS 0 µg/mL vs. HS 120 µg/mL	32.076	6.335	0.000	0.010	Yes
HS 0 µg/mL vs. HS 60 µg/mL	37.932	5.962	0.000	0.013	Yes
HS 0 µg/mL vs. HS 50 µg/mL	21.769	4.786	0.000	0.017	Yes
HS 0 µg/mL vs. HS 35 µg/mL	22.061	3.467	0.001	0.025	Yes
HS 0 µg/mL vs. HS 5 µg/mL	11.125	1.749	0.082	0.050	No

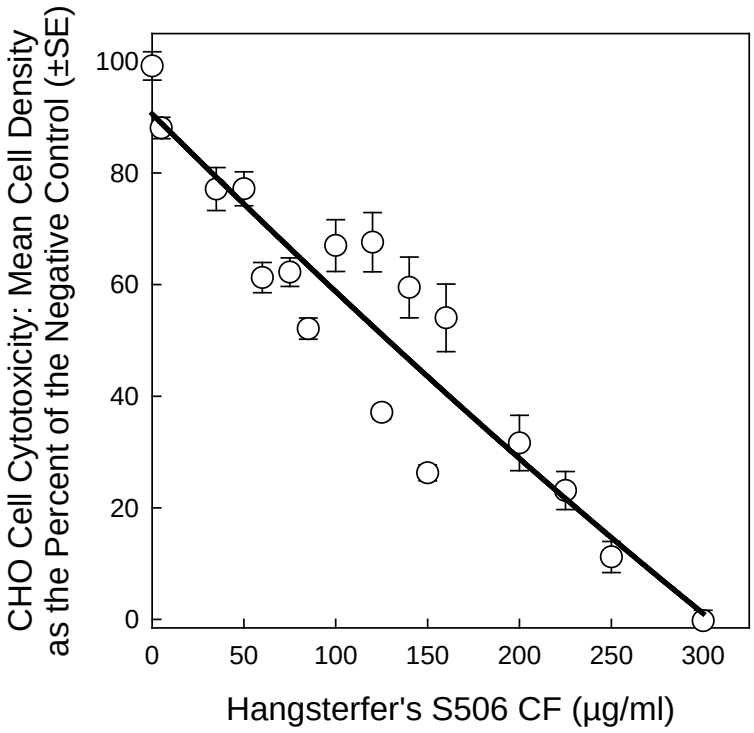

Figure 8. CHO cell chronic cytotoxicity concentration-response curve for Hangsterfer's S506.

Syntilo 9904

The summary data for the cytotoxicity assay for Syntilo 9904 is presented in Table 12 with the statistical analysis presented in Table 13 and the concentration-response curve in Figure 9.

Table 12. Summary of CHO cell cytotoxicity of Syntilo 9904.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 031006B 033106B 040406B Syntilo 9904 MWF CHO Cytotoxicity % NEGATIVE CONTROL SUMMARY																						
0	5	25	50	60	75	85	100	125	150	175	200	250	300	350	400	500	600	700	800	900	1000	µg/ml
106.90	120.04	117.15	119.15	112.25	87.53	49.44	54.12	70.16		23.39		30.51										
106.24	101.11	92.20	87.97	108.02	79.51	68.15	66.82	54.79		25.17		32.29										
111.58	103.34	94.88	90.65	85.08	67.04	55.23	62.36	55.23		43.88		34.52										
106.90	98.44	86.64	80.40	83.74	73.05	63.47	60.80	55.68		38.75		42.32										
96.88	84.41	98.44	92.87	78.17	75.95	66.82	47.44	38.98		34.97		39.64										
113.14	79.73	81.74	79.73	69.49	71.94	56.79	39.87	36.30		9.35		26.95										
92.87	65.26	75.06	78.17	51.45	52.34	64.37	46.55	21.38		9.80		36.75										
76.75		108.49	95.94				74.17		57.56		46.49	40.22	31.37	24.72	25.46	32.10						
94.10		81.18	95.57				63.10		77.49		66.05	37.27	40.22	29.89	15.13	27.31						
		90.04	86.35				75.65		73.43		61.62	55.35	48.34	38.38	31.73	19.56						
112.18		92.25	97.79				78.97		64.21		52.77	52.77	52.77	21.03	19.93	13.28						
95.94		91.88	70.48				74.17		70.48		54.61	45.39	34.32	29.15	25.09	23.99						
108.49		94.10	67.90				53.87		73.43		39.85	45.39	33.21	14.02	11.44	11.81						
116.24		98.52	85.24				73.80		79.34		74.54	38.38	31.00	25.46	32.10	29.89						
101.39							76.14				89.07		53.88		50.70	34.59	25.25	12.33	13.32	3.38	-6.16	
100.40							85.29				87.48		65.21		59.64	41.55	35.98	18.49	11.33	6.56	2.39	
113.52							76.14				77.73		68.59		58.65	43.54	33.80	32.60	15.51	7.95	4.17	
113.72							82.70				76.14		64.61		55.07	35.19	24.06	20.08	6.56	5.77	4.97	
100.99							75.55				73.96		57.46		50.50	38.77	27.63	30.22	0.00	-4.17	5.77	
93.84							87.08				75.15		43.34		55.47	25.25	23.66	11.53	-3.18	-7.36	0.20	
96.82							85.69				54.27		56.66		34.19	28.43	13.72	9.94	0.60	-7.75	-1.59	
20.00	7.00	14.00	14.00	7.00	7.00	7.00	21.00	7.00	7.00	7.00	14.00	14.00	14.00	7.00	14.00	14.00	7.00	7.00	7.00	7.00	7.00	number
102.94	93.19	93.04	87.73	84.03	72.48	60.61	68.58	47.50	70.85	26.47	66.41	39.84	48.64	26.09	37.51	28.95	26.30	19.31	6.31	0.62	1.39	Average
2.19	6.82	2.90	3.45	7.99	4.15	2.61	3.02	6.12	2.89	5.14	4.04	2.14	3.53	2.88	4.55	2.59	2.77	3.43	2.77	2.58	1.60	SE
9.80	18.04	10.84	12.89	21.14	10.98	6.90	13.85	16.19	7.64	13.59	15.13	8.00	13.20	7.62	17.04	9.68	7.34	9.07	7.32	6.83	4.24	SD

Table 13. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Syntilo 9904.

Data source: Data 9 in MWF 122706.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
Syntilo 0 µg/mL	20	0	102.944	9.803	2.192
Syntilo 5 µg/mL	7	0	93.191	18.042	6.819
Syntilo 25 µg/mL	14	0	93.040	10.842	2.898
Syntilo 50 µg/mL	14	0	87.730	12.892	3.445
Syntilo 60 µg/mL	7	0	84.028	21.136	7.989
Syntilo 75 µg/mL	7	0	72.479	10.983	4.151
Syntilo 85 µg/mL	7	0	60.611	6.902	2.609
Syntilo 100 µg/mL	21	0	68.584	13.854	3.023
Syntilo 125 µg/mL	7	0	47.502	16.193	6.121
Syntilo 150 µg/mL	7	0	70.849	7.640	2.888
Syntilo 175 µg/mL	7	0	26.472	13.590	5.136
Syntilo 200 µg/mL	14	0	66.410	15.133	4.044
Syntilo 250 µg/mL	14	0	39.839	8.001	2.138
Syntilo 300 µg/mL	14	0	48.640	13.203	3.529
Syntilo 350 µg/mL	7	0	26.094	7.618	2.879
Syntilo 400 µg/mL	14	0	37.507	17.036	4.553
Syntilo 500 µg/mL	14	0	28.946	9.681	2.587
Syntilo 600 µg/mL	7	0	26.299	7.339	2.774
Syntilo 700 µg/mL	7	0	19.313	9.075	3.430
Syntilo 800 µg/mL	7	0	6.305	7.321	2.767
Syntilo 900 µg/mL	7	0	0.625	6.830	2.581

Source of Variation	DF	SS	MS	F	P
Between Groups	20	187024.544	9351.227	61.208	<0.001
Residual	202	30861.004	152.777		
Total	222	217885.548			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Syntilo 0 µg/mL vs. Syntilo 900 µg/mL	102.320	18.850	2.133E-046	0.003	Yes
Syntilo 0 µg/mL vs. Syntilo 800 µg/mL	96.639	17.804	2.923E-043	0.003	Yes
Syntilo 0 µg/mL vs. Syntilo 500 µg/mL	73.998	17.180	2.263E-041	0.003	Yes
Syntilo 0 µg/mL vs. Syntilo 700 µg/mL	83.632	15.407	6.224E-036	0.003	Yes
Syntilo 0 µg/mL vs. Syntilo 400 µg/mL	65.437	15.193	2.865E-035	0.003	Yes
Syntilo 0 µg/mL vs. Syntilo 250 µg/mL	63.105	14.651	1.359E-033	0.003	Yes
Syntilo 0 µg/mL vs. Syntilo 350 µg/mL	76.851	14.158	4.601E-032	0.004	Yes
Syntilo 0 µg/mL vs. Syntilo 600 µg/mL	76.645	14.120	6.030E-032	0.004	Yes
Syntilo 0 µg/mL vs. Syntilo 175 µg/mL	76.473	14.088	7.563E-032	0.004	Yes
Syntilo 0 µg/mL vs. Syntilo 300 µg/mL	54.304	12.608	2.892E-027	0.005	Yes
Syntilo 0 µg/mL vs. Syntilo 125 µg/mL	55.442	10.214	5.186E-020	0.005	Yes
Syntilo 0 µg/mL vs. Syntilo 100 µg/mL	34.360	8.897	3.269E-016	0.006	Yes
Syntilo 0 µg/mL vs. Syntilo 200 µg/mL	36.534	8.482	4.667E-015	0.006	Yes
Syntilo 0 µg/mL vs. Syntilo 85 µg/mL	42.333	7.799	3.266E-013	0.007	Yes
Syntilo 0 µg/mL vs. Syntilo 150 µg/mL	32.096	5.913	0.0000000141	0.009	Yes
Syntilo 0 µg/mL vs. Syntilo 75 µg/mL	30.466	5.613	0.0000000652	0.010	Yes
Syntilo 0 µg/mL vs. Syntilo 50 µ µg/mL	15.215	3.532	0.000510	0.013	Yes
Syntilo 0 µg/mL vs. Syntilo 60 µg/mL	18.916	3.485	0.000604	0.017	Yes
Syntilo 0 µg/mL vs. Syntilo 25 µg/mL	9.904	2.300	0.0225	0.025	Yes
Syntilo 0 µg/mL vs. Syntilo 5 µg/mL	9.753	1.797	0.0739	0.050	No

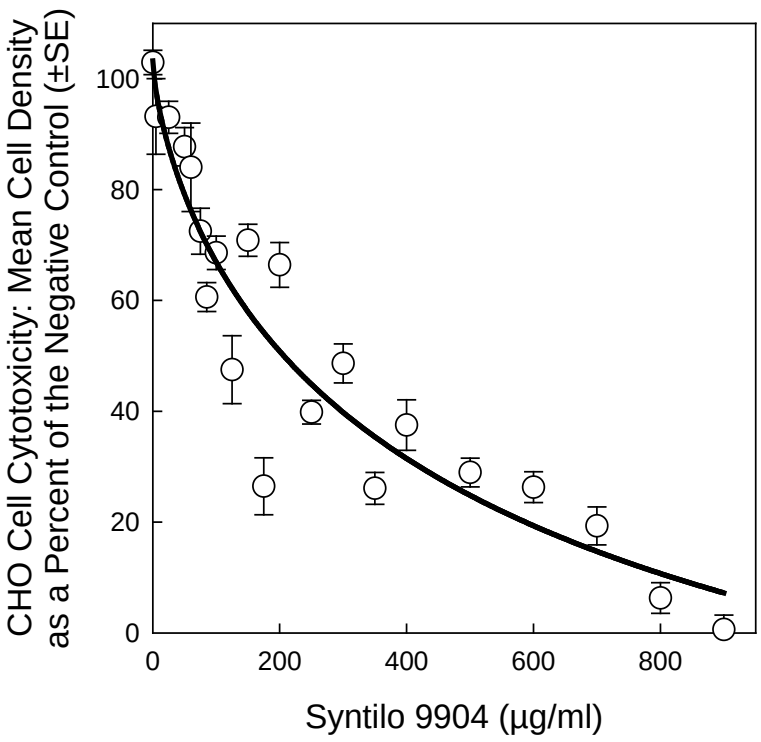

Figure 9. CHO cell chronic cytotoxicity concentration-response curve for Syntilo 9904.

TrimE 20bnd

The summary data for the CHO cell cytotoxicity assay for TrimE 20bnd is presented in Table 14 and the statistical analysis is presented in Table 15. Figure 10 presents the cytotoxicity concentration-response curve for the MWF.

Table 14. Summary of CHO cell cytotoxicity of TrimE 20bnd.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 010405, 031505MPJP, 041205JPMP TrimE20BND MWF CHO Cytotoxicity % NEGATIVE CONTROL												
0	5	25	35	50	60	75	85	100	125	150	250	µg/ml
0	1	5	7	10	12	15	17	20	25	30	50	µg/well
117.53	102.30	84.20		79.31							-4.02	
110.92	86.78	79.60		59.48							-2.87	
82.47	70.11	67.53		64.08							0.86	
72.70	74.71	62.07		80.46							0.29	
89.37	92.53	76.44		57.47							0.29	
101.72	80.46	68.68		61.21							-2.87	
125.00	85.34	89.66		49.71							-3.45	
99.14	101.72	93.68		44.54							-4.60	
117.53	78.61	69.33	86.34	101.55	66.24	52.32	43.81	37.63	29.90	38.14		
110.92	80.41	77.32	66.75	82.73	51.55	54.90	42.01	43.81	31.96	45.36		
82.47	88.14	70.62	76.80	77.06	57.47	50.77	46.39	42.01	35.82	39.43		
72.70	89.43	89.43	82.22	80.93	58.51	56.70	44.85	51.55	35.82	36.60		
89.37	95.88	71.13	73.71	78.87	68.04	63.14	39.18	43.56	39.43	31.44		
101.72	84.54	75.77	65.46	60.05	58.51	46.39	46.65	43.04	29.64	37.11		
125.00	99.23	91.24	88.40	84.79	86.34	68.81	47.68	54.38	37.11	43.56		
99.14	79.90	68.04	91.49	52.84	61.86	54.64	40.46	46.39	40.46	31.44		
94.33	165.52		131.32	81.90	65.23	48.56	52.87	31.03	20.69	12.64	-2.59	
87.89	128.45		90.80	69.83	70.98	38.22	52.87	29.60	16.09	4.31	8.62	
91.24	111.49		74.71	54.02	45.69	43.68	23.28	29.31	8.33	11.21	6.03	
83.51	102.30		72.41	58.05	52.87	36.49	29.60	20.98	13.22	9.77	2.01	
100.26	119.25		77.30	59.48	50.29	38.51	29.02	25.57	25.00	11.21	6.90	
92.78	97.99		72.70	47.99	57.76	35.92	33.33	22.70	26.15	15.52	0.86	
112.63	125.00		94.54	73.28	60.06	44.25	42.24	27.01	20.40	14.94	5.75	
136.34	114.37		82.18	62.93	50.57	38.51	32.76	21.26	14.08	13.79	8.62	
120.98												
96.26												
84.48												
83.05												
101.72												
89.94												
113.79												
109.48												
32	24	16	16	24	16	16	16	16	16	16	16	number
99.89	98.10	77.17	82.95	67.61	60.12	48.24	40.44	35.62	26.51	24.78	1.24	Average
2.82	4.36	2.46	3.90	2.92	2.48	2.45	2.17	2.75	2.54	3.54	1.15	SE

Table 15. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of TrimE 20bnd.

Data source: Data 6 in MWF 092506.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
TrimE20 0 µg/mL	32	0	99.887	15.931	2.816
TrimE20 5 µg/mL	24	0	98.103	21.347	4.358
TrimE20 25 µg/mL	16	0	77.170	9.838	2.459
TrimE20 35 µg/mL	16	0	82.948	15.617	3.904
TrimE20 50 µg/mL	24	0	67.606	14.312	2.922
TrimE20 60 µg/mL	16	0	60.122	9.915	2.479
TrimE20 75 µg/mL	16	0	48.239	9.816	2.454
TrimE20 85 µg/mL	16	0	40.438	8.663	2.166
TrimE20 100 µg/mL	16	0	35.615	10.989	2.747
TrimE20 125 µg/mL	16	0	26.508	10.140	2.535
TrimE20 150 µg/mL	16	0	24.780	14.174	3.543
TrimE20 250 µg/mL	16	0	1.239	4.613	1.153

Source of Variation	DF	SS	MS	F	P
Between Groups	11	211143.040	19194.822	104.604	<0.001
Residual	212	38901.984	183.500		
Total	223	250045.024			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
TrimE20 0 µg/mL vs. TrimE20 250 µg/mL	98.648	23.784	9.410E-062	0.005	Yes
TrimE20 0 µg/mL vs. TrimE20 150 µg/mL	75.107	18.108	6.472E-045	0.005	Yes
TrimE20 0 µg/mL vs. TrimE20 125 µg/mL	73.379	17.692	1.271E-043	0.006	Yes
TrimE20 0 µg/mL vs. TrimE20 100 µg/mL	64.272	15.496	1.019E-036	0.006	Yes
TrimE20 0 µg/mL vs. TrimE20 85 µg/mL	59.449	14.333	5.000E-033	0.007	Yes
TrimE20 0 µg/mL vs. TrimE20 75 µg/mL	51.648	12.452	4.486E-027	0.009	Yes
TrimE20 0 µg/mL vs. TrimE20 60 µg/mL	39.765	9.587	2.589E-018	0.010	Yes
TrimE20 0 µg/mL vs. TrimE20 50 µg/mL	32.281	8.825	4.119E-016	0.013	Yes
TrimE20 0 µg/mL vs. TrimE20 25 µg/mL	22.717	5.477	0.000000122	0.017	Yes
TrimE20 0 µg/mL vs. TrimE20 35 µg/mL	16.939	4.084	0.0000627	0.025	Yes
TrimE20 0 µg/mL vs. TrimE20 5 µg/mL	1.784	0.488	0.626	0.050	No

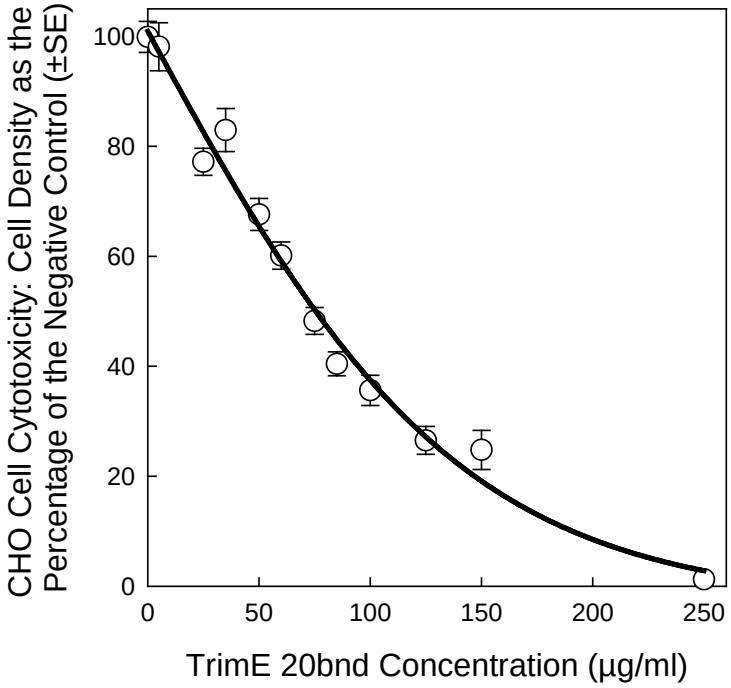

Figure 10. CHO cell chronic cytotoxicity concentration-response curve for TrimE 20bnd.

Trim Sol

The summary data for the cytotoxicity assay for Trim Sol is presented in Table 16 with the statistical analysis presented in Table 17 and the concentration-response curve in Figure 11.

Table 16. **Summary of CHO cell cytotoxicity of TrimSol.**
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 062105JPAG, 070505JP TrimSol MWF CHO Cytotoxicity PERCENT OF THE NEGATIVE CONTROL SUMMARY											
0	5	35	50	60	75	85	100	125	150	250	µg/ml
0	1	7	10	12	15	17	20	25	30	50	µg/well
121.80	121.45	111.42	96.54	72.66	80.28	38.41	34.26	25.95	16.26	6.57	
121.45	97.58	102.08	90.31	69.90	51.21	40.14	39.45	26.64	11.42	3.81	
98.96	102.08	87.54	75.09	62.63	53.98	43.25	37.02	19.03	10.38	3.81	
89.27	97.23	80.97	62.28	55.71	61.25			14.88	10.03	1.73	
98.96	88.24	90.66	77.16	49.48	56.40	68.86	23.88	14.53	9.69	-1.38	
86.85	82.01	91.35	65.74	60.55	47.75	40.48	44.64	16.26	10.38	5.54	
103.11	98.27	99.65	75.43	69.55	74.74	74.05	58.82	29.76	13.84	6.57	
78.20	91.35	83.04	57.44	55.02	63.67	67.47	48.10	19.38	11.76	5.19	
111.11	85.42	98.61	86.11	66.67	52.78	61.11	31.25	18.75	-0.69	-6.94	
95.83	82.64	72.92	56.94	59.03	41.67	43.75	22.92	-2.08	-4.86	13.19	
73.61	96.53	68.75	45.14	61.11	39.58	31.94	14.58	-3.47	-9.72	-11.11	
97.22	89.58	67.36	55.56	48.61	49.31	21.53	9.72	0.69	-9.72	-14.58	
96.53	82.64	68.75	62.50	52.78	47.22	27.78	22.92	-1.39	-4.17	-9.72	
98.61	76.39	61.81	49.31	37.50	27.08	22.22	16.67	-2.08	-9.72	-10.42	
140.97	97.92	83.33	67.36	65.28	34.03	41.67	36.11	14.58	6.25	4.86	
86.81	125.69	81.94	46.53	40.97	17.36	23.61	22.22	4.17	-2.78	-10.42	
16.000	16.000	16.000	16.000	16.000	16.000	15.000	15.000	16.000	16.000	16.000	number
99.96	94.69	84.39	66.84	57.97	49.89	43.09	30.84	12.23	3.65	-0.83	Average
4.293	3.369	3.546	3.872	2.557	4.054	4.483	3.507	2.826	2.322	2.102	SE
17.170	13.478	14.184	15.489	10.228	16.217	17.362	13.582	11.305	9.288	8.408	SD

Table 17. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Trim Sol.

Data source: Data 1 in Notebook 1

Group Name	N	Missing	Mean	Std Dev	SEM
TS 0 µg/mL	16	0	99.957	17.170	4.293
TS 5 µg/mL	16	0	94.688	13.478	3.369
TS 35 µg/mL	16	0	84.387	14.184	3.546
TS 50 µg/mL	16	0	66.840	15.489	3.872
TS 60 µg/mL	16	0	57.965	10.228	2.557
TS 75 µg/mL	16	0	49.894	16.217	4.054
TS 85 µg/mL	15	0	43.085	17.362	4.483
TS 100 µg/mL	15	0	30.837	13.582	3.507
TS 125 µg/mL	16	0	12.225	11.305	2.826
TS 150 µg/mL	16	0	3.647	9.288	2.322
TS 250 µg/mL	16	0	-0.832	8.408	2.102

Source of Variation	DF	SS	MS	F	P
Between Groups	10	201083.335	20108.334	107.992	<0.001
Residual	163	30350.954	186.202		
Total	173	231434.290			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
TS 0 µg/mL vs. TS 250 µg/mL	100.788	20.891	0.000	0.005	Yes
TS 0 µg/mL vs. TS 150 µg/mL	96.310	19.963	0.000	0.006	Yes
TS 0 µg/mL vs. TS 125 µg/mL	87.732	18.185	0.000	0.006	Yes
TS 0 µg/mL vs. TS 100 µg/mL	69.120	14.094	0.000	0.007	Yes
TS 0 µg/mL vs. TS 85 µg/mL	56.872	11.597	0.000	0.009	Yes
TS 0 µg/mL vs. TS 75 µg/mL	50.063	10.377	0.000	0.010	Yes
TS 0 µg/mL vs. TS 60 µg/mL	41.992	8.704	0.000	0.013	Yes
TS 0 µg/mL vs. TS 50 µg/mL	33.117	6.864	0.000	0.017	Yes
TS 0 µg/mL vs. TS 35 µg/mL	15.570	3.227	0.002	0.025	Yes
TS 0 µg/mL vs. TS 5 µg/mL	5.269	1.092	0.276	0.050	No

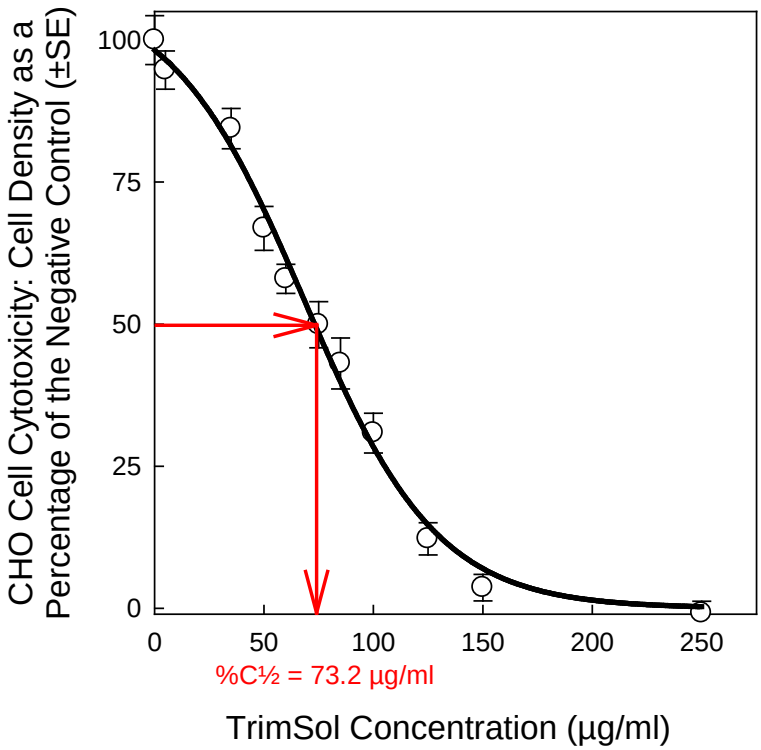

Figure 11. CHO cell chronic cytotoxicity concentration-response curve for TrimSol.

Vita Edge

The summary data for the cytotoxicity assay for Vita Edge is presented in Table 18 with the statistical analysis presented in Table 19 and the concentration-response curve in Figure 12.

Table 18. Summary of CHO cell cytotoxicity of Vita Edge. The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 070805JPAG 072205JPAG VitaEdge MWF CHO Cytotoxicity % NEGATIVE CONTROL																
0	5	10	20	25	30	35	40	50	60	75	85	100	125	150	250	µg/ml
0	1	2	4	5	6	7	8	10	12	15	17	20	25	30	50	µg/well
106.47	95.42	73.32	66.85	59.03	57.41	42.86	48.52	37.74	13.48	-9.43						
93.73	102.43	95.15	72.51	69.00	77.90	51.21	50.94	35.58	4.85	-15.09						
94.12	101.89	81.67	73.85	75.47	80.59	60.92	52.02	43.94	14.02	-14.02						
88.43	108.63	94.61	86.79	77.09	71.70	67.65	54.72	23.45	5.39	-13.21						
91.18	121.29	90.57	96.50	84.64	69.00	73.32	53.64	29.65	9.43	-15.36						
101.57	108.63	96.50	73.32	77.90	71.16	61.46	46.36	12.13	-4.58	-14.82						
119.02	104.85	94.34	86.79	98.11	64.96	53.91	47.17	32.88	11.86	-9.70						
104.90	115.63	77.90	69.81	57.95		42.86	30.46	23.18	-3.50	-12.13						
102.43	104.29	102.15	89.27	99.57	93.13	66.95	65.67	99.57	43.78	4.72						
92.18	86.70	72.96	59.23	76.39	61.80	61.37	79.83	64.38	29.61	-9.44						
74.39	85.41	81.12	68.67	100.43	73.82	86.27	111.16	53.22	29.61	0.86						
83.83	76.82	70.82	75.97	84.55	83.69	89.27	93.56	56.65	30.90	-1.72						
112.94	89.02	70.39	65.24	71.67	130.90	100.86	81.97	46.78	29.18	3.86						
97.84	89.02	73.39	60.09	84.55	93.13	81.12	73.82	47.21	12.45	-0.86						
128.57	97.84	81.97	75.11	78.54	81.97	116.31	53.65	73.39	28.76	-11.59						
108.09	110.39	61.80	56.22	87.98	54.51	58.37	32.19	44.21	24.46	0.86						
115.45	100.20					69.41		38.43	18.63	-0.98	8.63	11.57	0.78	1.37	3.92	
112.45	101.76					65.29		48.82	16.27	5.29	7.25	10.59	-0.39	0.00	2.75	
100.43	90.78					70.20		63.92	40.20	-0.59	9.22	16.67	0.98	3.14	0.20	
85.84	92.16					69.41		67.65	24.31	-3.53	5.10	-2.75	-0.20	0.20	3.53	
94.85						73.14		67.45	38.43	7.65	8.43	0.20	-0.98	1.37	-1.37	
83.26						86.47		63.14	55.29	10.59	9.41	10.20	-5.69	-2.94	-2.94	
111.59						76.08		56.86	46.08	23.73	6.86	3.92	1.96	1.18	-1.76	
95.28						80.20		62.75	50.00	13.73	-1.76	-4.12	-6.08	-4.31	-4.51	
24	20	16	16	16	15	24	16	24	24	24	8	8	8	8	8	number
99.95	99.16	82.41	73.51	80.18	77.71	71.04	60.98	49.71	23.70	-2.55	6.64	5.78	-1.20	0.00	-0.02	Average
2.63	2.48	2.95	2.87	3.17	4.83	3.50	5.48	3.95	3.36	2.11	1.30	2.67	1.07	0.87	1.11	SE
12.87	11.09	11.80	11.47	12.70	18.71	17.13	21.92	19.34	16.47	10.34	3.68	7.56	3.03	2.46	3.15	SD

Table 19. **Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Vita Edge.**

Data source**:** Data 2 in MWF 073105.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
VE 0 µg/mL	24	0	99.951	12.873	2.628
VE 5 µg/mL	20	0	99.158	11.086	2.479
VE 10 µg/mL	16	0	82.415	11.797	2.949
VE 20 µg/mL	16	0	73.513	11.467	2.867
VE 25 µg/mL	16	0	80.180	12.700	3.175
VE 30 µg/mL	15	0	77.712	18.715	4.832
VE 35 µg/mL	24	0	71.037	17.133	3.497
VE 40 µg/mL	16	0	60.980	21.925	5.481
VE 50 µg/mL	24	0	49.707	19.340	3.948
VE 60 µg/mL	24	0	23.705	16.473	3.363
VE 75 µg/mL	24	0	-2.550	10.338	2.110

Source of Variation	DF	SS	MS	F	P
Between Groups	10	220685.599	22068.560	94.074	<0.001
Residual	208	48793.957	234.586		
Total	218	269479.556			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
VE 0 µg/mL vs. VE 75 µg/mL	102.501	23.183	0.000	0.005	Yes
VE 0 µg/mL vs. VE 60 µg/mL	76.246	17.245	0.000	0.006	Yes
VE 0 µg/mL vs. VE 50 µg/mL	50.244	11.364	0.000	0.006	Yes
VE 0 µg/mL vs. VE 40 µg/mL	38.971	7.884	0.000	0.007	Yes
VE 0 µg/mL vs. VE 35 µg/mL	28.914	6.540	0.000	0.009	Yes
VE 0 µg/mL vs. VE 20 µg/mL	26.438	5.348	0.000	0.010	Yes
VE 0 µg/mL vs. VE 30 µg/mL	22.239	4.411	0.000	0.013	Yes
VE 0 µg/mL vs. VE 25 µg/mL	19.771	4.000	0.000	0.017	Yes
VE 0 µg/mL vs. VE 10 µg/mL	17.536	3.547	0.000	0.025	Yes
VE 0 µg/mL vs. VE 5 µg/mL	0.793	0.171	0.864	0.050	No

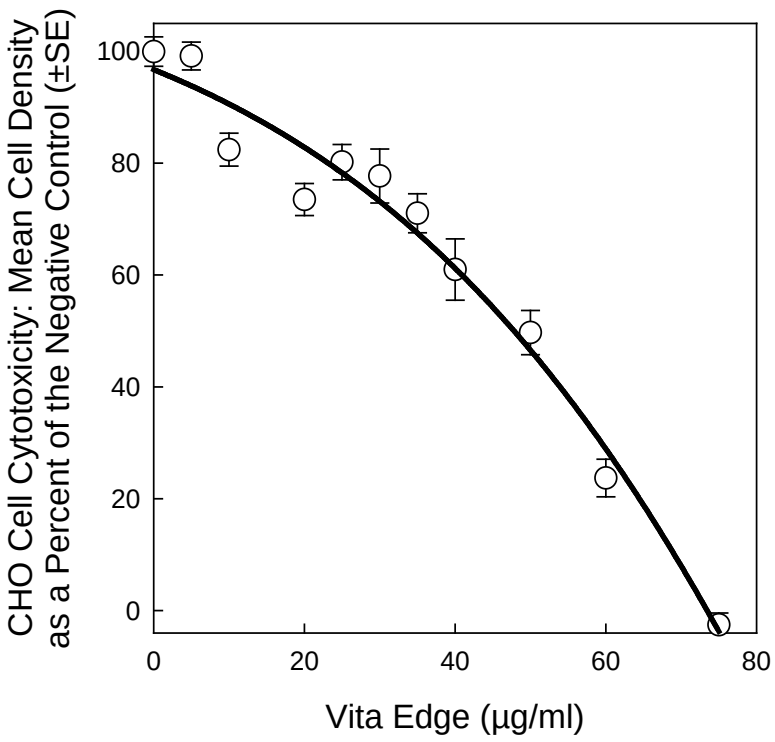

Figure 12. CHO cell chronic cytotoxicity concentration-response curve for Vita Edge.

Alusol AU39

The summary data for the CHO cell cytotoxicity assay for Alusol AU39 is presented in Table 20 and the statistical analysis is presented in Table 21. Figure 13 presents the cytotoxicity concentration-response curve for Alusol AU39.

Table 20. **Summary of CHO cell cytotoxicity of Alusol AU39.**
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 040706aJP, 041406aJP, 041806aJP MWF CHO Cytotoxicity SUMMARY % NEGATIVE CONTROL Alusol AU39														
0	5	25	50	60	75	85	100	125	150	175	200	225	250	µg/ml
0	1	5	10	12	15	17	20	25	30	35	40	45	50	µg/well
100.58	89.36	67.70	30.17	15.67	15.47	11.99	2.71	-0.77		-5.42			-14.89	
96.13	78.92	50.87	16.05	16.05	15.28	3.48	-0.19	-8.70		-8.32			-10.06	
104.84	83.56	65.96	28.43	10.25	11.61	2.71	1.16	-7.74		-11.99			-11.03	
111.41	92.46	59.96	37.14	6.19	9.67	1.35	1.35	-5.22		-13.54			-10.64	
91.68	74.85	58.99	32.30	5.61	9.48	-2.51	-1.74	-6.38		-9.28			-14.31	
90.52	76.60	54.16	30.17	10.25	16.05	16.05	0.19	-4.64		-10.44			-12.96	
111.80	90.72	49.90	18.76	6.38	7.35	11.61	3.48	-3.29		-5.42			-2.51	
93.04	88.20	38.30	0.39	-4.26	-4.45	-7.35	-12.19	-14.12		-15.67			-13.93	
96.76		87.78	44.14		34.91		11.47	7.98	-2.00	-7.73	-5.49	-8.48	-10.72	
90.52		76.06	38.65		22.44		6.48	1.00	-3.49	-7.48	-6.98	-8.23	-7.23	
101.75		59.10	24.19		19.20		6.73	0.75	-0.75	-4.49	-7.73	-8.98	-7.73	
110.22		53.87	39.90		10.22		1.75	-21.95	-4.99	-8.73	-12.97	-11.22	-16.21	
96.51		70.07	55.61		6.98		6.98	-2.24	-2.24	0.50	-10.22	-5.74	-7.48	
111.22		59.60	16.46		16.46		-6.73	2.00	-9.73	-6.23	-10.47	-12.72	-9.23	
107.48		79.05	34.41		15.71		14.71	14.21	-5.24	3.99	-4.99	-4.74	2.99	
85.04		73.07	34.16		0.50		0.50	0.75	-19.70	-8.98	-7.73	-6.23	-3.24	
16	8	16	16	8	16	8	16	16	8	16	8	8	16	number
99.97	84.33	62.78	30.06	8.27	12.93	4.67	2.29	-3.02	-6.02	-7.45	-8.32	-8.29	-9.32	Average
2.18	2.42	3.14	3.25	2.30	2.25	2.81	1.61	2.09	2.18	1.22	0.96	0.96	1.28	SE
8.72	6.83	12.56	13.01	6.49	8.98	7.96	6.44	8.36	6.18	4.86	2.71	2.73	5.10	SD

Table 21. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Alusol AU39.

Data source: Data 12 in MWF 040107.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
Alusol 0 µg/mL	16	0	99.969	8.723	2.181
Alusol 5 µg/mL	8	0	84.333	6.831	2.415
Alusol 25 µg/mL	16	0	62.778	12.560	3.140
Alusol 50 µg/mL	16	0	30.060	13.011	3.253
Alusol 60 µg/mL	8	0	8.269	6.492	2.295
Alusol 75 µg/mL	16	0	12.931	8.980	2.245
Alusol 85 µg/mL	8	0	4.666	7.961	2.815
Alusol 100 µg/mL	16	0	2.292	6.445	1.611
Alusol 125 µg/mL	16	0	-3.024	8.359	2.090
Alusol 150 µg/mL	8	0	-6.016	6.178	2.184
Alusol 175 µg/mL	16	0	-7.452	4.865	1.216
Alusol 200 µg/mL	8	0	-8.323	2.712	0.959
Alusol 225 µg/mL	8	0	-8.292	2.728	0.964
Alusol 250 µg/mL	16	0	-9.324	5.100	1.275

Source of Variation	DF	SS	MS	F	P
Between Groups	13	227614.625	17508.817	255.328	<0.001
Residual	162	11108.948	68.574		
Total	175	238723.572			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Alusol 0 µg/mL vs. Alusol 250 µg/mL	109.293	37.330	1.773E-081	0.004	Yes
Alusol 0 µg/mL vs. Alusol 175 µg/mL	107.421	36.691	2.170E-080	0.004	Yes
Alusol 0 µg/mL vs. Alusol 125 µg/mL	102.992	35.178	9.333E-078	0.005	Yes
Alusol 0 µg/mL vs. Alusol 100 µg/mL	97.677	33.362	1.773E-074	0.005	Yes
Alusol 0 µg/mL vs. Alusol 200 µg/mL	108.292	30.201	1.943E-068	0.006	Yes
Alusol 0 µg/mL vs. Alusol 225 µg/mL	108.261	30.192	2.021E-068	0.006	Yes
Alusol 0 µg/mL vs. Alusol 75 µg/mL	87.038	29.729	1.692E-067	0.007	Yes
Alusol 0 µg/mL vs. Alusol 150 µg/mL	105.985	29.557	3.732E-067	0.009	Yes
Alusol 0 µg/mL vs. Alusol 85 µg/mL	95.302	26.578	5.960E-061	0.010	Yes
Alusol 0 µg/mL vs. Alusol 60 µg/mL	91.700	25.573	9.282E-059	0.013	Yes
Alusol 0 µg/mL vs. Alusol 50 µg/mL	69.909	23.878	6.130E-055	0.017	Yes
Alusol 0 µg/mL vs. Alusol 25 µg/mL	37.191	12.703	4.270E-026	0.025	Yes
Alusol 0 µg/mL vs. Alusol 5 µg/mL	15.636	4.361	0.0000230	0.050	Yes

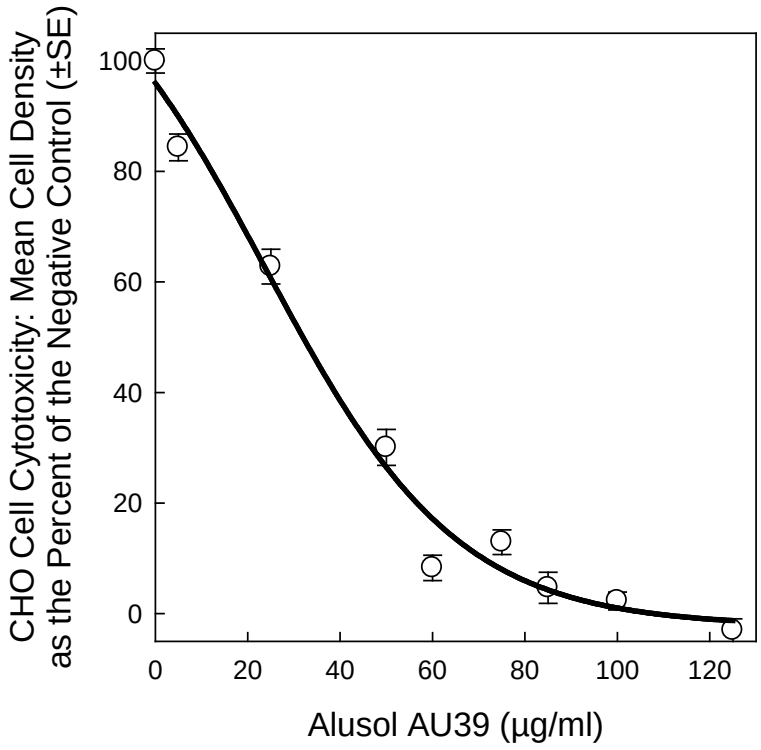

Figure 13. CHO cell chronic cytotoxicity concentration-response curve for Alusol AU39.

Castrol Clearedge 6536

The summary data for the cytotoxicity assay for Castrol Clearedge 6536 is presented in Table 22 with the statistical analysis presented in Table 23 and the concentration-response curve in Figure 14.

Table 22. Summary of CHO cell cytotoxicity of Castrol Clearedge 6536.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment MWF Castrol 6536 CHO Cytotoxicity % NEGATIVE CONTROL SUMMARY											
0	5	15	25	30	40	50	60	75	100	125	µg/ml
0	1	3	5	6	8	10	12	15	20	25	µg/well
83.85	71.14	36.29	76.86	58.86	57.71	50.29	8.86	2.29	-2.86	-9.14	
91.75	30.29	26.57	54.00	52.29	35.14	33.43	3.71	-2.00	-1.71	-2.29	
111.68	39.43	31.71	83.14	50.86	35.14	41.43	0.86	-12.86	-10.57	-3.71	
114.43	38.29	31.14	63.71	54.00	71.14	51.43	-10.29	9.71	-11.71	6.86	
104.81	58.57	28.57	64.86	52.86	39.14	33.43	-7.14	-17.14	-16.29	-9.71	
105.50	44.00	38.00	61.43	64.86	35.14	29.14	-6.00	-9.14	-7.71	-6.57	
109.62	65.43	34.57	47.43	63.71	21.14	31.43	-9.14	-3.71	-6.29	-10.00	
78.35	27.71	31.14	59.43	48.29	14.00	51.71	-15.71	-5.14	-10.00	-9.14	
71.43	82.92	65.55	56.30	53.63	42.75	19.56	15.46	8.78	-2.00	-0.10	
75.71	92.65	62.31	67.37	43.80	38.93	25.48	19.37	4.96	-0.29	1.43	
102.57	88.26	68.70	68.89	66.22	50.00	22.33	19.37	6.39	3.82	2.58	
110.29	84.45	62.69	75.19	74.05	52.00	20.80	10.11	10.69	3.44	-1.81	
126.29	90.27	67.18	69.66	59.64	50.57	15.55	4.10	9.45	-1.91	-3.24	
105.43	88.36	75.10	71.76	56.20	55.82	19.94	6.39	13.55	-0.57	-0.38	
118.57	73.09	73.95	56.39	49.14	50.57	21.56	7.25	11.35	7.06	2.77	
90.57	63.84	56.68	51.34	44.18	56.11	20.04	4.68	-6.77	-0.19	-0.57	
111.55											
103.34											
100.29											
95.52											
100.00											
91.22											
105.15											
92.84											
24	16	16	16	16	16	16	16	16	16	16	number
100.03	64.92	49.38	64.23	55.79	44.08	30.47	3.24	1.27	-3.61	-2.69	Average
1.42	5.68	4.59	2.47	2.09	3.60	3.06	2.64	2.38	1.58	1.27	SE
13.64	22.71	18.38	9.88	8.34	14.39	12.24	10.56	9.54	6.32	5.07	SD

Table 23. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Castrol Clearedge 6536.

Data source: Data 13 in MWF 040107.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
Cast6536 0 µg/mL	24	0	100.032	13.642	2.785
Cast6536 5 µg/mL	16	0	64.918	22.714	5.679
Cast6536 15 µg/mL	16	0	49.385	18.376	4.594
Cast6536 25 µg/mL	16	0	64.234	9.879	2.470
Cast6536 30 µg/mL	16	0	55.785	8.342	2.085
Cast6536 40 µg/mL	16	0	44.083	14.390	3.598
Cast6536 50 µg/mL	16	0	30.472	12.244	3.061
Cast6536 60 µg/mL	16	0	3.242	10.558	2.639
Cast6536 75 µg/mL	16	0	1.275	9.540	2.385
Cast6536 100 µg/mL	16	0	-3.612	6.324	1.581
Cast6536 125 µg/mL	16	0	-2.690	5.070	1.268

Source of Variation	DF	SS	MS	F	P
Between Groups	10	218130.471	21813.047	130.263	<0.001
Residual	173	28969.601	167.454		
Total	183	247100.072			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Cast6536 0 µg/mL vs. Cast6536 100 µg/mL	103.644	24.816	6.875E-059	0.005	Yes
Cast6536 0 µg/mL vs. Cast6536 125 µg/mL	102.722	24.595	2.296E-058	0.006	Yes
Cast6536 0 µg/mL vs. Cast6536 75 µg/mL	98.757	23.646	4.409E-056	0.006	Yes
Cast6536 0 µg/mL vs. Cast6536 60 µg/mL	96.789	23.175	6.247E-055	0.007	Yes
Cast6536 0 µg/mL vs. Cast6536 50 µg/mL	69.560	16.655	8.742E-038	0.009	Yes
Cast6536 0 µg/mL vs. Cast6536 40 µg/mL	55.949	13.396	1.560E-028	0.010	Yes
Cast6536 0 µg/mL vs. Cast6536 15 µg/mL	50.647	12.127	6.875E-025	0.013	Yes
Cast6536 0 µg/mL vs. Cast6536 30 µg/mL	44.246	10.594	1.574E-020	0.017	Yes
Cast6536 0 µg/mL vs. Cast6536 25 µg/mL	35.798	8.571	5.541E-015	0.025	Yes
Cast6536 0 µg/mL vs. Cast6536 5 µg/mL	35.113	8.407	1.498E-014	0.050	Yes

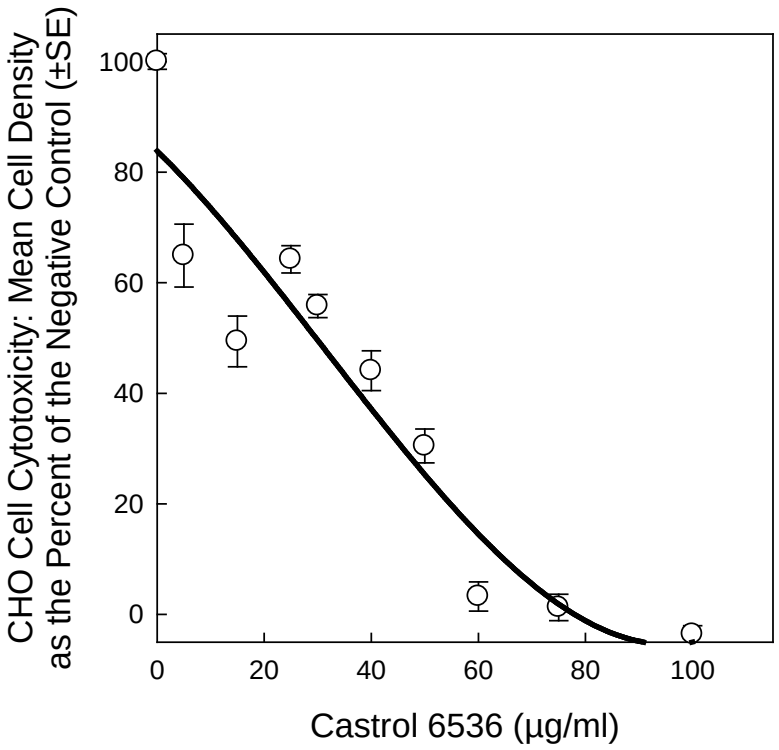

Figure 14. CHO cell chronic cytotoxicity concentration-response curve for Castrol Clearedge 6536.

IRMCO Cutting Fluid Product A

The summary data for the cytotoxicity assay for IRMCO Cutting Fluid Product A is presented in Table 24 with the statistical analysis presented in Table 25 and the concentration-response curve in Figure 15.

Table 24. Summary of CHO cell cytotoxicity of IRMCO Cutting Fluid Product A.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 040706bJP, 041406bJP, 041806bJP MWF CHO Cytotoxicity SUMMARY % NEG CONTROL IRMCO CUT PRODUCT A																						
0	5	25	50	60	75	85	100	125	175	200	250	300	350	400	450	500	550	600	700	800	µg/ml
0	1	5	10	12	15	17	20	25	35	40	50	60	70	80	90	100	110	120	140	160	µg/well
80.81	110.35	91.16	89.90	84.34	75.25	54.80	50.51	66.16	47.47	42.68											
71.21	103.54	103.79	95.20	90.15	84.85	83.08	70.96	73.48	60.35	46.97											
82.58	112.63	115.15	119.19	103.54	100.25	94.95	82.83	73.99	69.19	59.34											
95.71	106.57	107.58	108.59	101.01	84.85	91.67	80.05	66.41	66.41	55.30											
120.96	102.27	127.27	115.15	110.61	96.72	98.99	79.55	67.68	58.33	47.73											
131.06	110.86	107.83	102.78	100.00	71.21	69.44	72.73	62.12	51.01	38.89											
129.80	103.28	116.67	104.55	96.46	88.89	67.17	58.08	48.48	43.18	51.77											
85.37			82.66				85.91			67.75	65.58	61.25		52.85		18.16		-7.05	5.96	-6.78	
102.71			90.24				81.57			85.09	69.11	76.15		69.38		44.44		15.18	15.45	-4.07	
99.73			125.47				76.15			111.92	86.99	97.29		67.21		46.34		18.70	3.25	1.63	
103.52			138.21				82.93			123.04	83.74	113.01		76.69		47.97		27.37	6.23	-0.54	
108.67			86.18				71.27			72.09	72.36	72.90		68.83		27.10		7.32	-0.81	-7.05	
83.85			88.89				79.95			87.53	86.18	89.97		75.88		46.88		14.63	9.21	-1.36	
91.75							77.78			97.29	70.73	52.57		77.51		23.58		-0.27	4.61	0.81	
111.68			94.85				84.19			90.72	53.61	83.51	70.79	65.29		51.89	50.52				
114.43			75.26				82.13			111.00	68.38	89.35	61.86	50.52	49.83	19.24	32.99				
104.81			99.31				100.34			100.34	100.69	80.41	70.79	66.32	61.51	37.80	26.80				
105.50			87.97				91.07			86.94	73.88	89.35	66.67	51.55	49.14	32.99	23.71				
109.62			106.53				102.75			92.10	83.51	88.32	73.54	53.26	55.33	46.05	15.81				
78.35			82.13				83.51			89.35	58.42	65.98	56.70	45.36	57.39	26.12	16.49				
			101.03				114.78			76.63	67.35	76.63	68.73	59.45	57.73	31.96	6.19				
			95.53				84.19			60.14	60.48	58.76	49.48	35.40	45.02	29.21	20.62				
20.00	7.00	7.00	21.00	7.00	7.00	7.00	22.00	7.00	7.00	22.00	15.00	15.00	8.00	15.00	7.00	15.00	8.00	7.00	7.00	7.00	number
100.61	107.07	109.92	99.51	98.02	86.00	80.01	81.51	65.48	56.57	77.03	73.40	79.70	64.82	61.03	53.71	35.32	24.14	10.84	6.27	-2.48	Average
3.77	1.59	4.29	3.38	3.28	3.97	6.25	2.91	3.25	3.67	5.20	3.26	4.12	2.92	3.23	2.21	2.92	4.71	4.43	1.92	1.33	SE
16.86	4.21	11.35	15.50	8.69	10.51	16.54	13.63	8.59	9.72	24.40	12.63	15.97	8.25	12.49	5.84	11.31	13.33	11.72	5.08	3.52	SD

Table 25. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of IRMCO Cutting Fluid Product A.

Data source: Data 11 in MWF 040107.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
Irmco 0 µg/mL	20	0	100.606	16.861	3.770
Irmco 5 µg/mL	7	0	107.071	4.205	1.589
Irmco 25 µg/mL	7	0	109.921	11.353	4.291
Irmco 50 µg/mL	21	0	99.506	15.502	3.383
Irmco 60 µg/mL	7	0	98.016	8.690	3.284
Irmco 75 µg/mL	7	0	86.003	10.513	3.974
Irmco 85 µg/mL	7	0	80.014	16.537	6.250
Irmco 100 µg/mL	22	0	81.509	13.628	2.905
Irmco 125 µg/mL	7	0	65.476	8.590	3.247
Irmco 175 µg/mL	7	0	56.566	9.717	3.673
Irmco 20 0µg/mL	22	0	77.028	24.395	5.201
Irmco 250 µg/mL	15	0	73.401	12.633	3.262
Irmco 300 µg/mL	15	0	79.696	15.971	4.124
Irmco 350 µg/mL	8	0	64.820	8.245	2.915
Irmco 400 µg/mL	15	0	61.033	12.494	3.226
Irmco 450 µg/mL	7	0	53.706	5.841	2.208
Irmco 500 µg/mL	15	0	35.315	11.312	2.921
Irmco 550 µg/mL	8	0	24.141	13.330	4.713
Irmco 600 µg/mL	7	0	10.840	11.719	4.429
Irmco 700 µg/mL	7	0	6.272	5.083	1.921
Irmco 800 µg/mL	7	0	-2.478	3.525	1.332

Source of Variation	DF	SS	MS	F	P
Between Groups	20	200591.931	10029.597	49.451	<0.001
Residual	217	44011.536	202.818		
Total	237	244603.467			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Irmco 0 µg/mL vs. Irmco 800 µg/mL	103.084	16.482	4.046E-040	0.003	Yes
Irmco 0 µg/mL vs. Irmco 700 µg/mL	94.334	15.083	1.224E-035	0.003	Yes
Irmco 0 µg/mL vs. Irmco 600 µg/mL	89.766	14.353	2.720E-033	0.003	Yes
Irmco 0 µg/mL vs. Irmco 500 µg/mL	65.291	13.422	2.637E-030	0.003	Yes
Irmco 0 µg/mL vs. Irmco 550 µg/mL	76.465	12.835	1.986E-028	0.003	Yes
Irmco 0 µg/mL vs. Irmco 400 µg/mL	39.573	8.135	3.156E-014	0.003	Yes
Irmco 0 µg/mL vs. Irmco 450 µg/mL	46.900	7.499	1.626E-012	0.004	Yes
Irmco 0 µg/mL vs. Irmco 175 µg/mL	44.040	7.042	2.470E-011	0.004	Yes
Irmco 0 µg/mL vs. Irmco 350 µg/mL	35.786	6.007	0.00000000789	0.004	Yes
Irmco 0 µg/mL vs. Irmco 125 µg/mL	35.130	5.617	0.0000000592	0.005	Yes
Irmco 0 µg/mL vs. Irmco 250 µg/mL	27.205	5.593	0.0000000669	0.005	Yes
Irmco 0 µg/mL vs. Irmco 200 µg/mL	23.578	5.359	0.000000213	0.006	Yes
Irmco 0 µg/mL vs. Irmco 100 µg/mL	19.097	4.340	0.0000218	0.006	Yes
Irmco 0 µg/mL vs. Irmco 300 µg/mL	20.910	4.299	0.0000260	0.007	Yes
Irmco 0 µg/mL vs. Irmco 85 µg/mL	20.592	3.292	0.00116	0.009	Yes
Irmco 0 µg/mL vs. Irmco 75 µg/mL	14.603	2.335	0.0205	0.010	No
Irmco 0 µg/mL vs. Irmco 25 µg/mL	9.315	1.489	0.138	0.013	No
Irmco 0 µg/mL vs. Irmco 5 µg/mL	6.465	1.034	0.302	0.017	No
Irmco 0 µg/mL vs. Irmco 60 µg/mL	2.590	0.414	0.679	0.025	No
Irmco 0 µg/mL vs. Irmco 50 µg/mL	1.100	0.247	0.805	0.050	No

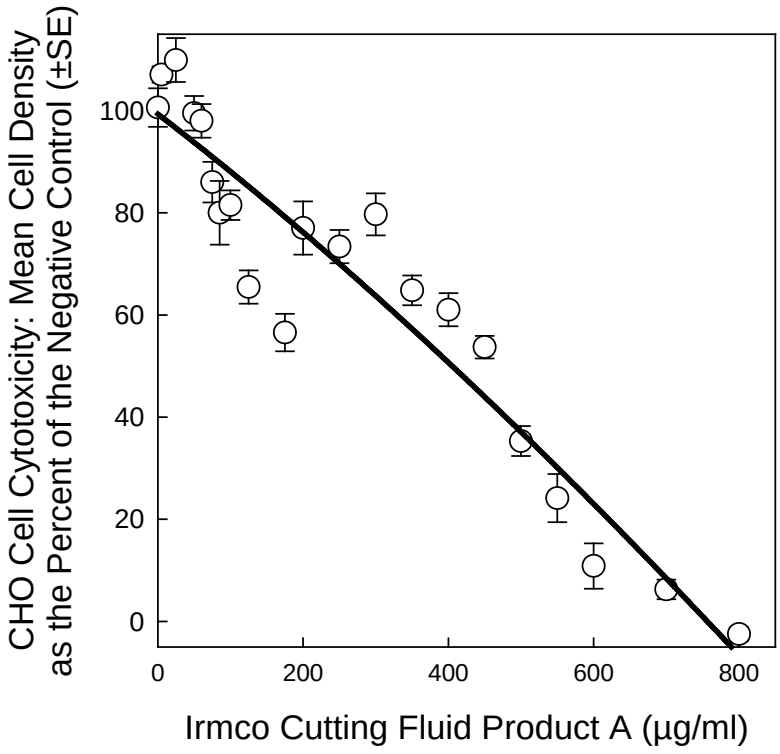

Figure 15. CHO cell chronic cytotoxicity concentration-response curve for IRMCO Cutting Fluid Product A.

MWF Components

Bunsan 77

The summary data for the CHO cell cytotoxicity assay for Busan 77 MWF component is presented in Table 26 and the statistical analysis is presented in Table 27. Figure 16 presents the cytotoxicity concentration-response curve for Busan 77.

Table 26. **Summary of CHO cell cytotoxicity of Busan 77.**
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 090806, 091206, 092106AJP BUSAN77 MWF CHO Cytotoxicity % NEGATIVE CONTROL SUMMARY											
0	5	10	20	25	35	45	50	60	75	100	µg/ml
0	1	2	4	5	7	9	10	12	15	20	µg/well
89.92	73.01			46.70			2.91		-9.97	-12.77	
66.74	89.70			67.75			2.46		-2.46	-10.19	
83.43	76.60			65.29			7.84		2.91	-5.38	
100.22	88.91			69.65			13.66		-2.69	-7.28	
110.97	96.30			85.78			9.41		1.57	-3.25	
105.71	100.90			88.58			20.72		1.57	-5.49	
125.42	104.37			104.70			27.66		7.39	-4.93	
117.81	82.42						28.33		9.29	-6.05	
115.81	102.72	116.29	77.48	70.61	53.99	44.25	23.48	10.38	0.00	-12.46	
92.17	103.67	116.45	85.30	81.63	48.88	41.21	9.27	-2.40	-1.60	-10.38	
97.92	108.63	98.40	87.38	101.60	55.91	44.41	17.25	-3.67	-3.99	-9.74	
101.44	106.55	94.89	104.95	74.92	68.69	36.26	7.03	-7.51	-6.71	-11.18	
104.15	102.88	94.09	74.60	69.81	75.08	49.68	1.44	-4.95	-16.45	-15.65	
91.85	89.94	90.42	77.32	73.32	54.15	17.89	1.12	-13.90	-13.26	-13.90	
96.33	92.33	85.94	84.66	62.30	50.80	41.21	-4.47	2.72	-12.46	-11.98	
153.94	54.55	105.45	31.52	31.52	17.58	27.27	-11.52	-9.70	-13.33	4.24	
66.06	60.61	56.97	36.97	31.52	5.45	3.03	-16.97	-9.09	-15.15	-9.09	
63.64	53.33	40.61	33.94	26.06	17.58	-6.06	-20.61	-15.76	-12.12	-9.09	
61.21	51.52	41.21	27.88	32.12	9.70	-3.64	-5.45	-35.15	-31.52	-8.48	
127.88	38.18	101.82	50.91	49.09	-0.61		-29.09	-24.24	-22.42	-20.61	
48.48	34.55	17.58	9.70	-7.88	-3.03		-27.88	-24.85	-29.09	-28.48	
150.30	36.97	73.94	28.48	7.88	-4.24	-30.30	-14.55	-16.36	-17.58	-14.55	
127.88	104.85	105.45	33.33	13.33	1.21	-6.06	-24.85	-22.42	-21.82	-22.42	
23.00	23.00	15.00	15.00	22.00	15.00	13.00	23.00	15.00	23.00	23.00	number
99.97	80.59	82.63	56.29	56.65	30.08	19.94	0.75	-11.79	-9.13	-10.83	Average
5.73	5.21	7.79	7.56	6.51	7.40	7.17	3.63	3.05	2.31	1.42	SE
27.50	24.99	30.16	29.28	30.53	28.66	25.86	17.41	11.83	11.08	6.82	SD

Table 27. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Busan 77.

Data source: Data 14 in MWF 092207.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
Busan77 0 µg/mL	23	0	99.969	27.502	5.735
Busan77 5 µg/mL	23	0	80.585	24.985	5.210
Busan77 10 µg/mL	15	0	82.634	30.162	7.788
Busan77 20 µg/mL	15	0	56.295	29.279	7.560
Busan77 25 µg/mL	22	0	56.648	30.532	6.509
Busan77 35 µg/mL	15	0	30.076	28.662	7.400
Busan77 45 µg/mL	13	0	19.936	25.855	7.171
Busan77 50 µg/mL	23	0	0.748	17.412	3.631
Busan77 60 µg/mL	15	0	-11.794	11.828	3.054

Source of Variation	DF	SS	MS	F	P
Between Groups	8	227093.336	28386.667	42.784	<0.001
Residual	155	102839.629	663.481		
Total	163	329932.966			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Busan77 0 µg/mL vs. Busan77 60 µg/mL	111.763	13.074	8.470E-027	0.006	Yes
Busan77 0 µg/mL vs. Busan77 50 µg/mL	99.222	13.063	9.063E-027	0.007	Yes
Busan77 0 µg/mL vs. Busan77 45 µg/mL	80.034	8.955	1.003E-015	0.009	Yes
Busan77 0 µg/mL vs. Busan77 35 µg/mL	69.893	8.176	9.818E-014	0.010	Yes
Busan77 0 µg/mL vs. Busan77 25 µg/mL	43.321	5.640	0.0000000787	0.013	Yes
Busan77 0 µg/mL vs. Busan77 20 µg/mL	43.675	5.109	0.000000940	0.017	Yes
Busan77 0 µg/mL vs. Busan77 5 µg/mL	19.384	2.552	0.0117	0.025	Yes
Busan77 0 µg/mL vs. Busan77 10 µg/mL	17.335	2.028	0.0443	0.050	Yes

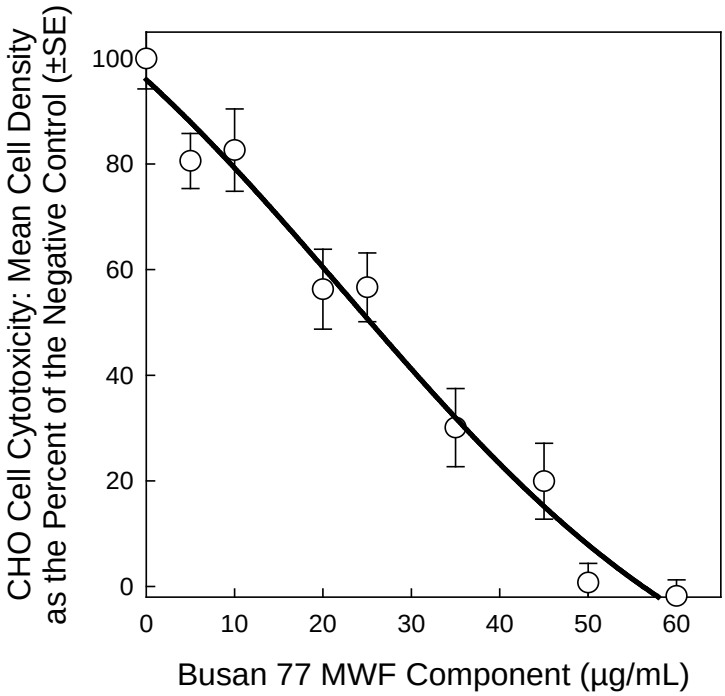

Figure 16. CHO cell chronic cytotoxicity concentration-response curve for Busan 77.

UCON EMPL-48

The summary data for the cytotoxicity assay for UCON EMPL-48 MWF component is presented in Table 28 with the statistical analysis presented in Table 29 and the concentration-response curve in Figure 17.

Table 28. **Summary of CHO cell cytotoxicity of UCON EMPL-48.**
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 090806, 091206, 092106 UCON EMPL-48 MWF Component MWF CHO Cytotoxicity % NEGATIVE CONTROL SUMMARY															
0	5	25	50	75	100	125	150	175	200	225	250	300	350	400	µg/ml
0	1	5	10	15	20	25	30	35	40	45	50	60	70	80	µg/well
89.00	92.76	83.65	61.79	50.51	42.11	27.50	27.50	27.79	21.85		-7.38				
90.45	83.07	92.91	89.87	80.61	58.61	47.76	41.39	36.90	29.96		-1.59				
91.32	93.92	88.57	94.21	74.53	76.70	60.35	55.72	36.61	37.92		7.38				
106.37	81.48	103.18	98.26	93.63	89.44	54.99	51.52	46.02	33.43		19.25				
102.89	103.18	116.64	97.40	104.49	84.37	72.50	52.68	48.91	47.32		33.86				
112.59	100.58	109.12	105.35	119.83	82.05	58.76	55.14	44.28	44.72		35.46				
107.38	107.38	113.75	106.66	84.52	83.07	72.94	66.43	63.24	61.36		52.53				
88.94	98.12	90.01	78.58	55.43	63.53	54.27	45.88	41.39	44.28		53.11				
94.18		122.84		92.91	87.23	72.34	58.44	62.84	62.98	50.07	44.26	40.85			
127.80		160.14		126.67	128.37	106.52	93.62	95.32	71.21	47.38	47.80	43.55			
101.13		160.14		117.16	121.70	89.65	82.98	82.27	75.60	58.87	50.64	47.94			
97.59		139.29		118.16	101.28	82.55	104.82	72.91	54.18	69.22	61.28	35.60			
95.46		129.36		110.78	86.52	87.09	87.09	61.99	63.12	56.31	60.28	38.16			
95.18		124.11		103.83	97.87	77.59	68.37	58.30	41.84	44.11	48.51	36.60			
109.41		104.68		92.34	88.65	68.51	67.94	66.52	43.55	49.79	41.84	42.27			
63.53		97.06			108.24		49.41	47.06	62.94	54.71	47.06	74.71	44.12	32.94	
68.24		106.47			85.88		80.59	95.29	66.47	64.71	61.18	40.00	80.00	41.18	
70.59		82.94			76.47		82.35	54.12	64.12	59.41	54.12	38.82	43.53	50.59	
73.53		77.65			97.65		84.71	94.12	62.35	68.82	55.29	41.18	39.41	33.53	
120.00		97.65			77.65		93.53	132.94	64.12	77.06	54.71	35.88	38.82	27.65	
151.76		114.12			77.65		131.18	72.35	82.35	69.41	48.24	37.65	38.24	25.29	
142.35		110.59			128.82		87.06	78.82	72.94	72.35	57.65	43.53	30.59	41.76	
		57.06			47.06		87.06	51.18	133.53	67.65	91.76	59.41	29.41	36.47	
22	8	23	8	15	23	15	23	23	23	15	23	15	8	8	number
99.99	95.06	107.91	91.52	95.03	86.56	68.89	71.97	63.96	58.35	60.66	44.23	43.74	43.01	36.18	Average
4.74	3.25	5.15	5.28	5.92	4.65	4.99	4.94	5.10	4.73	2.61	4.62	2.69	5.61	2.91	SE
22.25	9.20	24.71	14.94	22.93	22.32	19.32	23.69	24.46	22.68	10.10	22.14	10.44	15.87	8.22	SD

Table 29. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of UCON EMPL-48.

Data source: Data 15 in MWF 092207.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
UCON-48 0 µg/mL	22	0	99.986	22.250	4.744
UCON-48 5 µg/mL	8	0	95.062	9.200	3.253
UCON-48 25 µg/mL	23	0	107.910	24.712	5.153
UCON-48 50 µg/mL	8	0	91.516	14.935	5.280
UCON-48 75 µg/mL	15	0	95.025	22.926	5.920
UCON-48 100 µg/mL	23	0	86.562	22.323	4.655
UCON-48 125 µg/mL	15	0	68.888	19.319	4.988
UCON-48 150 µg/mL	23	0	71.973	23.694	4.941
UCON-48 175 µg/mL	23	0	63.964	24.461	5.100
UCON-48 200 µg/mL	23	0	58.354	22.681	4.729
UCON-48 225 µg/mL	15	0	60.657	10.096	2.607
UCON-48 250 µg/mL	23	0	44.227	22.140	4.617
UCON-48 300 µg/mL	15	0	43.743	10.436	2.695
UCON-48 350 µg/mL	8	0	43.015	15.867	5.610
UCON-48 400 µg/mL	8	0	36.176	8.217	2.905

Source of Variation	DF	SS	MS	F	P
Between Groups	14	121460.835	8675.774	20.092	<0.001
Residual	237	102335.528	431.795		
Total	251	223796.363			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
UCON-48 0 µg/mL vs. UCON-48 250 µg/mL	55.759	8.998	7.621E-017	0.004	Yes
UCON-48 0 µg/mL vs. UCON-48 300 µg/mL	56.243	8.083	3.231E-014	0.004	Yes
UCON-48 0 µg/mL vs. UCON-48 400 µg/mL	63.810	7.438	1.868E-012	0.004	Yes
UCON-48 0 µg/mL vs. UCON-48 200 µg/mL	41.632	6.718	0.000000000135	0.005	Yes
UCON-48 0 µg/mL vs. UCON-48 350 µg/mL	56.971	6.641	0.000000000211	0.005	Yes
UCON-48 0 µg/mL vs. UCON-48 175 µg/mL	36.022	5.813	0.0000000197	0.006	Yes
UCON-48 0 µg/mL vs. UCON-48 225 µg/mL	39.329	5.652	0.0000000453	0.006	Yes
UCON-48 0 µg/mL vs. UCON-48 150 µg/mL	28.013	4.521	0.00000974	0.007	Yes
UCON-48 0 µg/mL vs. UCON-48 125 µg/mL	31.099	4.469	0.0000122	0.009	Yes
UCON-48 0 µg/mL vs. UCON-48 100 µg/mL	13.424	2.166	0.0313	0.010	No
UCON-48 0 µg/mL vs. UCON-48 25 µg/mL	7.924	1.279	0.202	0.013	No
UCON-48 0 µg/mL vs. UCON-48 50 µg/mL	8.470	0.987	0.324	0.017	No
UCON-48 0 µg/mL vs. UCON-48 75 µg/mL	4.961	0.713	0.477	0.025	No
UCON-48 0 µg/mL vs. UCON-48 5 µg/mL	4.925	0.574	0.566	0.050	No

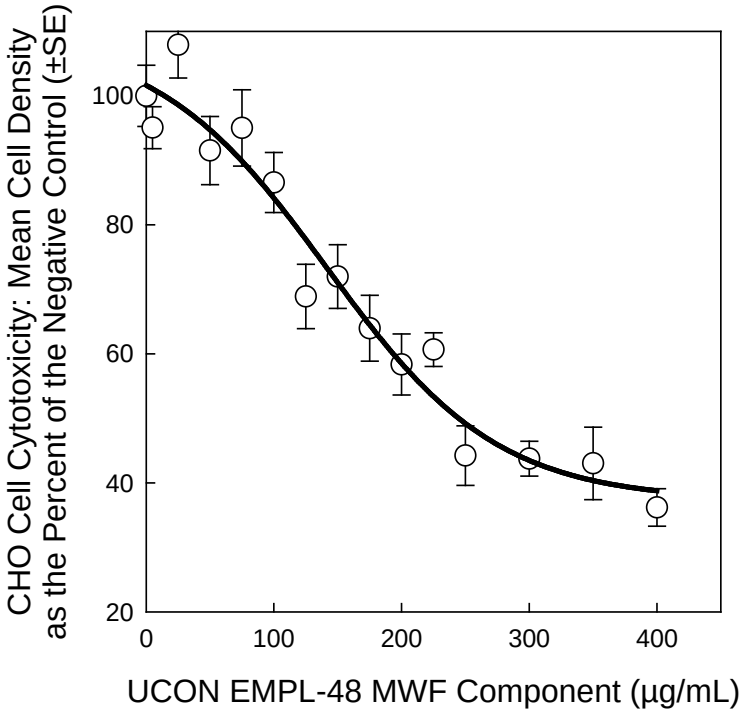

Figure 17. CHO cell chronic cytotoxicity concentration-response curve for UCON EMPL-48.

AMP95

The summary data for the CHO cell cytotoxicity assay for the MWF component AMP95 is presented in Table 30 and the statistical analysis is presented in Table 31. Figure 18 presents the cytotoxicity concentration-response curve for AMP95.

Table 30. **Summary of CHO cell cytotoxicity of AMP95.**
The cell density is a percentage of the concurrent negative control for each microplate.

Summary of Experiments with AMP95 MWF Component										
0	5	25	50	75	100	125	150	175	200	µg/mL
0	1	5	10	15	20	25	30	35	40	µg/well
99.73	108.94	56.37	65.04	65.04	61.79	53.39	46.07	47.15	31.71	
109.76	128.46	117.89	79.95	94.31	61.25	68.83	42.82	69.65	44.72	
92.14		112.20	92.68	77.78	69.92	64.50	64.77	33.33	31.17	
96.21	125.47	102.71	92.68	75.61	69.92	50.68	44.99	38.75	26.83	
96.75	131.71	92.41	78.05	90.24	75.07	72.63	61.25	40.11	19.51	
101.63	110.84	78.32	66.94	62.06	64.77	52.30	42.01	15.18	7.05	
105.96	92.95	105.96	77.78	62.06	60.16	62.87	33.60	38.21	13.01	
97.02	82.93	59.35	49.86	39.30	52.57	54.47	25.75	-0.81	12.74	
100.00		131.40	125.07	133.25	101.32	98.15	104.22	65.17	84.43	
101.06	106.33	114.25	130.61	149.34	106.86	105.28	83.91	62.01	40.11	
95.51	121.11	135.88	123.75	109.76	100.79	100.00	75.73	65.44	40.11	
98.42		136.41	121.37	107.39	98.42	89.97	80.21	54.62	32.19	
102.11	123.48	145.38	122.16	122.96	102.90	88.39	75.20	91.56	37.99	
99.74	111.35	115.83	115.57	122.69	88.65	115.83	82.59	90.24	26.65	
103.96	112.93	122.43	106.33	98.94	83.91	73.88	98.42	64.12	23.48	
99.21	86.54	84.43	82.06	107.92	87.86	82.59	98.68	26.39	22.96	
16	13	16	16	16	16	16	16	16	16	Number
99.95	111.00	106.95	95.62	94.91	80.38	77.11	66.26	50.07	30.91	Average
1.07	4.34	6.67	6.35	7.47	4.52	5.18	6.19	6.31	4.46	SE
4.27	15.66	26.68	25.41	29.87	18.09	20.71	24.77	25.25	17.85	SD

Table 31. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of AMP95.

Data source: Data 16 in MWF 092207.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
AMP95 0 µg/mL	16	0	99.949	4.271	1.068
AMP95 5 µg/mL	16	3	111.003	15.660	4.343
AMP95 25 µg/mL	16	0	106.951	26.683	6.671
AMP95 50 µg/mL	16	0	95.618	25.414	6.353
AMP95 75 µg/mL	16	0	94.915	29.871	7.468
AMP95 100 µg/mL	16	0	80.385	18.093	4.523
AMP95 125 µg/mL	16	0	77.110	20.708	5.177
AMP95 150 µg/mL	16	0	66.262	24.767	6.192
AMP95 175 µg/mL	16	0	50.069	25.254	6.314
AMP95 200 µg/mL	16	0	30.915	17.852	4.463

Source of Variation	DF	SS	MS	F	P
Between Groups	9	93898.513	10433.168	21.343	<0.001
Residual	147	71858.907	488.836		
Total	156	165757.420			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
AMP95 0 µg/mL vs. AMP95 200 µg/mL	69.034	8.831	2.855E-015	0.006	Yes
AMP95 0 µg/mL vs. AMP95 175 µg/mL	49.881	6.381	0.00000000216	0.006	Yes
AMP95 0 µg/mL vs. AMP95 150 µg/mL	33.687	4.310	0.0000298	0.007	Yes
AMP95 0 µg/mL vs. AMP95 125 µg/mL	22.839	2.922	0.00403	0.009	Yes
AMP95 0 µg/mL vs. AMP95 100 µg/mL	19.564	2.503	0.0134	0.010	No
AMP95 0 µg/mL vs. AMP95 5 µg/mL	11.054	1.339	0.183	0.013	No
AMP95 0 µg/mL vs. AMP95 25 µg/mL	7.002	0.896	0.372	0.017	No
AMP95 0 µg/mL vs. AMP95 75 µg/mL	5.034	0.644	0.521	0.025	No
AMP95 0 µg/mL vs. AMP95 50 µg/mL	4.331	0.554	0.580	0.050	No

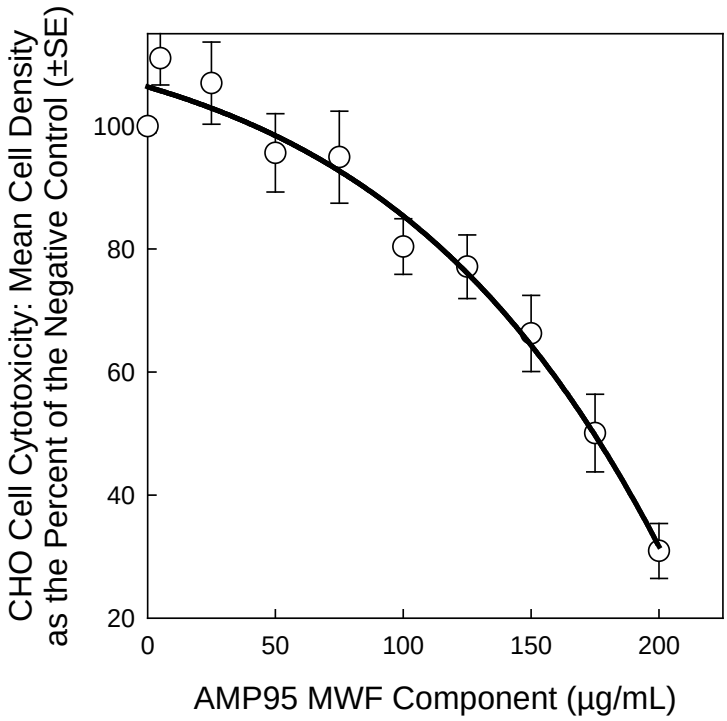

Figure 18. CHO cell chronic cytotoxicity concentration-response curve for AMP95.

UCON 50-HB-6

The summary data for the cytotoxicity assay for the MWF component UCON 50-HB-6 is presented in Table 32 with the statistical analysis presented in Table 33 and the concentration-response curve in Figure 19.

Table 32. **Summary of CHO cell cytotoxicity of UCON 50-HB-6.**
The cell density is a percentage of the concurrent negative control for each microplate.

0	5	25	50	75	100	125	150	175	200	225	250	300	350	400	µg/ml
0	1	5	10	15	20	25	30	35	40	45	50	60	70	80	µg/well
106.03	102.01	100.00	102.30	104.02	103.16	121.84	97.99	88.51	77.30		54.89	75.84	64.61	56.46	
95.40	106.32	109.20	108.62	115.52	96.26	97.70	95.69	92.53	73.56		66.09	72.19	67.70	64.61	
92.82	118.10	118.97	123.56	110.92	104.89	99.14	97.70	91.09	81.03		56.90	88.48	66.57	58.71	
101.15	118.68	115.23	98.56	102.87	95.69	104.02	94.54	87.07	72.70		52.01	81.74	71.07	55.06	
113.51	99.14	114.37	111.49	100.86	101.44	93.39	87.64	89.37	75.29		50.00	78.09	67.70	56.74	
97.13	90.80	106.32	103.16	98.28	101.72	83.91	94.83	77.87	62.64		50.57	73.88	64.04	57.87	
100.29	114.37	94.54	106.03	97.99	87.07	88.51	66.09	68.68	61.78		54.02	73.60	64.89	62.64	
93.39		135.39			135.96		110.96	109.83	88.48	92.98	80.06	57.02	54.78	50.56	
111.80		105.90			117.70		120.79	99.16	109.55	103.09	82.02				
84.27		104.21			122.47		119.66	101.12	95.22	85.96	79.78				
78.93		121.35			126.40		109.55	105.06	99.44	104.21	96.07				
97.75		125.00			115.17		112.92	107.02	110.39	85.96	81.18				
100.00		114.61			107.58		142.13	97.75	89.04	104.78	89.04				
101.69		120.51			109.83		112.92	96.07	85.96	100.28	87.64				
118.26		89.61			91.29		83.99	73.60	81.18	66.57	77.25				
107.87															
16	7	15	7	7	15	7	15	15	15	8	15	8	8	8	number
100.02	107.06	111.68	107.68	104.35	107.78	98.36	103.16	92.31	84.24	92.98	70.50	75.11	65.17	57.83	Average
2.56	3.97	3.11	3.09	2.48	3.51	4.66	4.69	3.10	3.83	4.66	4.16	3.19	1.68	1.54	SE
10.26	10.51	12.05	8.19	6.57	13.62	12.34	18.19	12.02	14.84	13.18	16.12	9.03	4.76	4.3	SD

Table 33. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of UCON 50-HB-6.

Data source: Data 17 in MWF 092207.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
HB-6 0 µg/mL	16	0	100.017	10.258	2.565
HB-6 5 µg/mL	7	0	107.061	10.515	3.974
HB-6 25 µg/mL	15	0	111.680	12.054	3.112
HB-6 50 µg/mL	7	0	107.677	8.195	3.097
HB-6 75 µg/mL	7	0	104.351	6.579	2.487
HB-6 100 µg/mL	15	0	107.776	13.628	3.519
HB-6 125 µg/mL	7	0	98.358	12.349	4.668
HB-6 150 µg/mL	15	0	103.160	18.191	4.697
HB-6 175 µg/mL	15	0	92.315	12.022	3.104
HB-6 200 µg/mL	15	0	84.239	14.847	3.834
HB-6 225 µg/mL	8	0	92.978	13.185	4.662
HB-6 250 µg/mL	15	0	70.501	16.125	4.163
HB-6 300 µg/mL	8	0	75.105	9.037	3.195
HB-6 350 µg/mL	8	0	65.169	4.769	1.686
HB-6 400 µg/mL	8	0	57.830	4.366	1.544

Source of Variation	DF	SS	MS	F	P
Between Groups	14	42234.440	3016.746	19.046	<0.001
Residual	151	23917.044	158.391		
Total	165	66151.484			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
HB-6 0 µg/mL vs. HB-6 400 µg/mL	42.187	7.741	1.315E-012	0.004	Yes
HB-6 0 µg/mL vs. HB-6 250 µg/mL	29.516	6.526	0.000000000967	0.004	Yes
HB-6 0 µg/mL vs. HB-6 350 µg/mL	34.849	6.395	0.00000000190	0.004	Yes
HB-6 0 µg/mL vs. HB-6 300 µg/mL	24.912	4.571	0.0000100	0.005	Yes
HB-6 0 µg/mL vs. HB-6 200 µg/mL	15.778	3.488	0.000637	0.005	Yes
HB-6 0 µg/mL vs. HB-6 25 µg/mL	11.662	2.578	0.0109	0.006	No
HB-6 0 µg/mL vs. HB-6 100 µg/mL	7.758	1.715	0.0883	0.006	No
HB-6 0 µg/mL vs. HB-6 175 µg/mL	7.702	1.703	0.0906	0.007	No
HB-6 0 µg/mL vs. HB-6 50 µg/mL	7.659	1.343	0.181	0.009	No
HB-6 0 µg/mL vs. HB-6 225 µg/mL	7.040	1.292	0.198	0.010	No
HB-6 0 µg/mL vs. HB-6 5 µg/mL	7.044	1.235	0.219	0.013	No
HB-6 0 µg/mL vs. HB-6 75 µg/mL	4.334	0.760	0.448	0.017	No
HB-6 0 µg/mL vs. HB-6 150 µg/mL	3.143	0.695	0.488	0.025	No
HB-6 0 µg/mL vs. HB-6 125 µg/mL	1.659	0.291	0.772	0.050	No

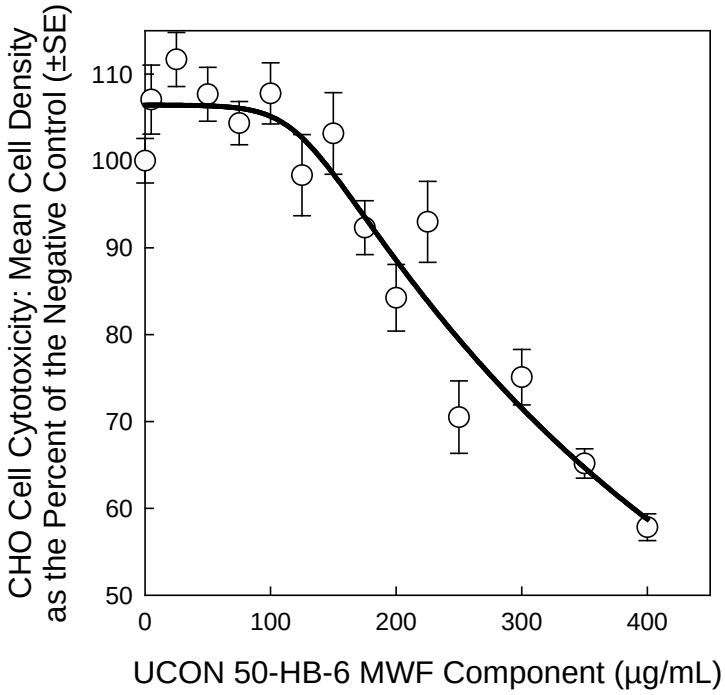

Figure 19. CHO cell chronic cytotoxicity concentration-response curve for UCON 50-HB-6.

Alkaterg T-IV

The summary data for the CHO cell cytotoxicity assay for the MWF component Alkaterg T-IV is presented in Table 34 and the statistical analysis is presented in Table 35. Figure 20 presents the cytotoxicity concentration-response curve for Alkaterg T-IV.

Table 34. **Summary of CHO cell cytotoxicity of Alkaterg T-IV.**
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 101006JP, 102406AJP, 120506AJP Alkaterg T-IV MWF CHO Cytotoxicity % NEGATIVE CONTROL									
0	1	2.5	5	10	15	20	25	50	µg/ml
0	0.2	0.5	1	2	3	4	5	10	µg/well
101.04	138.72	94.53	109.11				41.67	2.60	
92.19	109.57	105.92	88.80				23.18	3.91	
97.40	125.51		105.99				20.83	1.82	
104.17	147.61		94.53				10.16	-4.17	
113.80		125.74	102.08				10.16	3.91	
99.48	131.89	121.41	94.01				8.59	-1.04	
92.19	101.82	84.74	80.47				10.94	-2.08	
96.46	73.12	64.69	67.97				10.42	-3.39	
105.32			92.15	84.56	56.20	34.68	5.32	-7.85	
114.68			83.29	86.33	49.37	17.47	1.77	-8.10	
97.47			86.33	79.24	47.09	26.33	9.11	-8.10	
108.86			83.80	79.75	47.59	15.95	5.32	-4.56	
96.46			82.78	80.76	39.75	12.41	7.09	-6.33	
97.72			91.39	85.82	36.71	17.97	4.81	-5.57	
83.54			83.29	73.92	38.73	18.23	13.67	-2.28	
88.61			69.87	58.48	29.62	12.41	2.53		
107.97									
121.18									
93.17									
94.76									
102.73									
114.58									
76.54									
23.00	7.00	6.00	16.00	8.00	8.00	8.00	16.00	15.00	number
100.01	118.32	99.51	88.49	78.61	43.13	19.43	11.60	-2.75	Average
2.20	9.64	9.42	2.85	3.22	2.99	2.67	2.48	1.09	SE
10.56	25.50	23.08	11.40	9.11	8.45	7.55	9.91	4.24	SD

Table 35. Statistical analysis of the CHO cell cytotoxicity data of AlkatergT-IV.

Data source: Data 18 in MWF 010108.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
Alkaterg 0 µg/mL	23	0	100.014	10.563	2.203
Alkaterg 1 µg/mL	7	0	118.321	25.501	9.638
Alkaterg 2.5 µg/mL	6	0	99.506	23.083	9.424
Alkaterg 5 µg/mL	16	0	88.493	11.404	2.851
Alkaterg 10 µg/mL	8	0	78.608	9.106	3.219
Alkaterg 15 µg/mL	8	0	43.133	8.451	2.988
Alkaterg 20 µg/mL	8	0	19.430	7.545	2.668
Alkaterg 25 µg/mL	16	0	11.597	9.910	2.477
Alkaterg 50 µg/mL	15	0	-2.748	4.238	1.094

Source of Variation	DF	SS	MS	F	P
Between Groups	8	197745.762	24718.220	170.897	<0.001
Residual	98	14174.532	144.638		
Total	106	211920.294			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Alkaterg 0 µg/mL vs. Alkaterg 50 µg/mL	102.762	25.746	2.094E-045	0.006	Yes
Alkaterg 0 µg/mL vs. Alkaterg 25 µg/mL	88.416	22.583	1.264E-040	0.007	Yes
Alkaterg 0 µg/mL vs. Alkaterg 20 µg/mL	80.583	16.324	1.047E-029	0.009	Yes
Alkaterg 0 µg/mL vs. Alkaterg 15 µg/mL	56.881	11.523	6.257E-020	0.010	Yes
Alkaterg 0 µg/mL vs. Alkaterg 10 µg/mL	21.406	4.336	0.0000352	0.013	Yes
Alkaterg 0 µg/mL vs. Alkaterg 1 µg/mL	18.307	3.526	0.000642	0.017	Yes
Alkaterg 0 µg/mL vs. Alkaterg 5 µg/mL	11.521	2.943	0.00406	0.025	Yes
Alkaterg 0 µg/mL vs. Alkaterg 2.5 µg/mL	0.507	0.0920	0.927	0.050	No

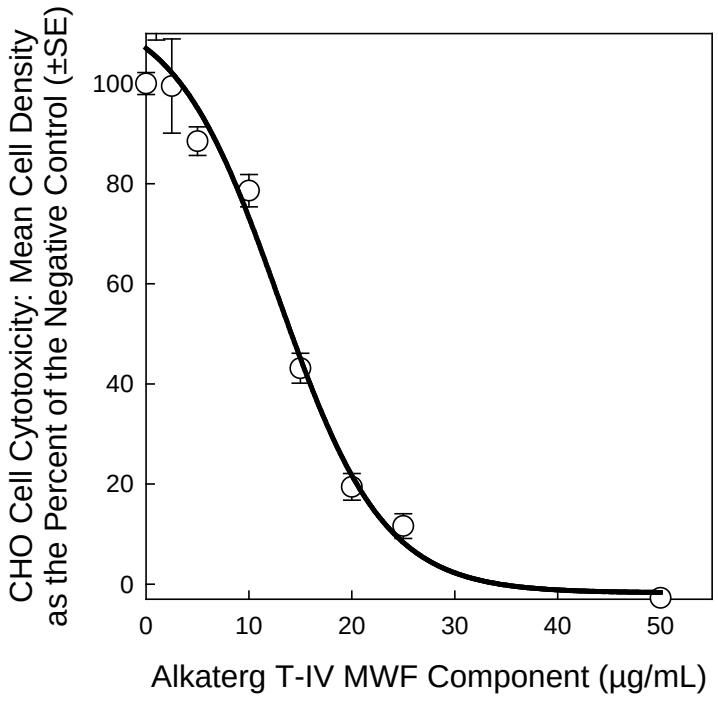

Figure 20. Concentration-response curve for the CHO cell chronic cytotoxicity of Alkaterg T-IV.

Actramide 202

The summary data for the cytotoxicity assay for Actramide 202 MWF component is presented in Table 36 with the statistical analysis presented in Table 37 and the concentration-response curve in Figure 21.

Table 36. Summary of CHO cell cytotoxicity of Actramide 202.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 102406BJP 120506BJP Actramide 202 MWF CHO Cytotoxicity % NEGATIVE CONTROL											
0	1	2.5	5	6	7	8	9	10	12.5	15	µg/ml
0	0.2	0.5	1	1.2	1.4	1.6	1.8	2	2.5	3	µg/well
95.17			128.26								
96.38			86.23								
90.58			102.66								
93.00			88.16								
101.69			107.49								
86.71			85.75								
124.40			89.61								
112.80											
95.39			91.13					36.52		4.26	
107.80			83.69					10.64		-0.35	
101.06			80.85					11.35		-1.06	
108.51			85.11					-0.71		-6.38	
101.42			89.36					7.09		1.42	
96.81			74.11					16.67		-5.67	
89.72			73.05					15.60		-0.35	
								2.84		-0.71	
88.51	131.03	152.71	117.57	102.63	139.90	82.59	73.89	59.28	57.80	8.70	
93.60	123.32	130.21	119.87	80.30	99.01	90.48	94.09	63.38	34.98	9.03	
107.22	93.43	101.64	79.64	87.52	74.55	93.60	93.60	69.13	26.77	1.64	
123.15	119.54	101.48	95.07	85.22	74.88	73.40	73.56	57.80	20.85	-3.28	
84.40	113.46	89.66	74.88	77.01	88.18	77.18	79.64	61.08	33.17	0.33	
105.75	90.48	76.68	76.19	78.98	65.02	55.50	57.64	57.96	12.81	2.79	
97.37	112.15	94.75	82.43	84.24	58.46	70.11	66.01	98.52	28.90	9.36	
22	7	7	21	7	7	7	7	15	7	15	number
100.07	111.92	106.73	91.01	85.13	85.71	77.55	76.92	37.81	30.75	1.31	Average
2.29	5.69	9.83	3.41	3.24	10.39	4.90	5.10	7.92	5.33	1.26	SE
10.76	15.04	26.00	15.65	8.56	27.48	12.97	13.49	30.66	14.11	4.89	SD

Table 37. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Actramide 202.

Data source: Data 19 in MWF 010108.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
Actramide 0 µg/mL	22	0	100.065	10.759	2.294
Actramide 1 µg/mL	7	0	111.916	15.041	5.685
Actramide 2.5 µg/mL		0	106.732	26.003	9.828
Actramide 5 µg/mL	21	0	91.006	15.645	3.414
Actramide 6 µg/mL	7	0	85.128	8.561	3.236
Actramide 7 µg/mL	7	0	85.714	27.485	10.388
Actramide 8 µg/mL	7	0	77.551	12.967	4.901
Actramide 9 µg/mL	7	0	76.918	13.488	5.098
Actramide 10 µg/mL	15	0	37.811	30.659	7.916
Actramide 12.5 µg/mL	7	0	30.753	14.110	5.333
Actramide 15 µg/mL	15	0	1.314	4.894	1.264

Source of Variation	DF	SS	MS	F	P
Between Groups	10	151779.456	15177.946	48.829	<0.001
Residual	111	34503.176	310.839		
Total	121	186282.632			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
Actramide 0 µg/mL vs. Actramide 15 µg/mL	98.751	16.728	4.080E-032	0.005	Yes
Actramide 0 µg/mL vs. Actramide 10 µg/mL	62.255	10.545	1.975E-018	0.006	Yes
Actramide 0 µg/mL vs. Actramide 12.5 µg/mL	69.312	9.059	5.209E-015	0.006	Yes
Actramide 0 µg/mL vs. Actramide 9 µg/mL	23.148	3.026	0.00308	0.007	Yes
Actramide 0 µg/mL vs. Actramide 8 µg/mL	22.514	2.943	0.00396	0.009	Yes
Actramide 0 µg/mL vs. Actramide 6 µg/mL	14.937	1.952	0.0534	0.010	No
Actramide 0 µg/mL vs. Actramide 7 µg/mL	14.351	1.876	0.0633	0.013	No
Actramide 0 µg/mL vs. Actramide 5 µg/mL	9.060	1.684	0.0949	0.017	No
Actramide 0 µg/mL vs. Actramide 1 µg/mL	11.851	1.549	0.124	0.025	No
Actramide 0 µg/mL vs. Actramide 2.5 µg/mL	6.667	0.871	0.385	0.050	No

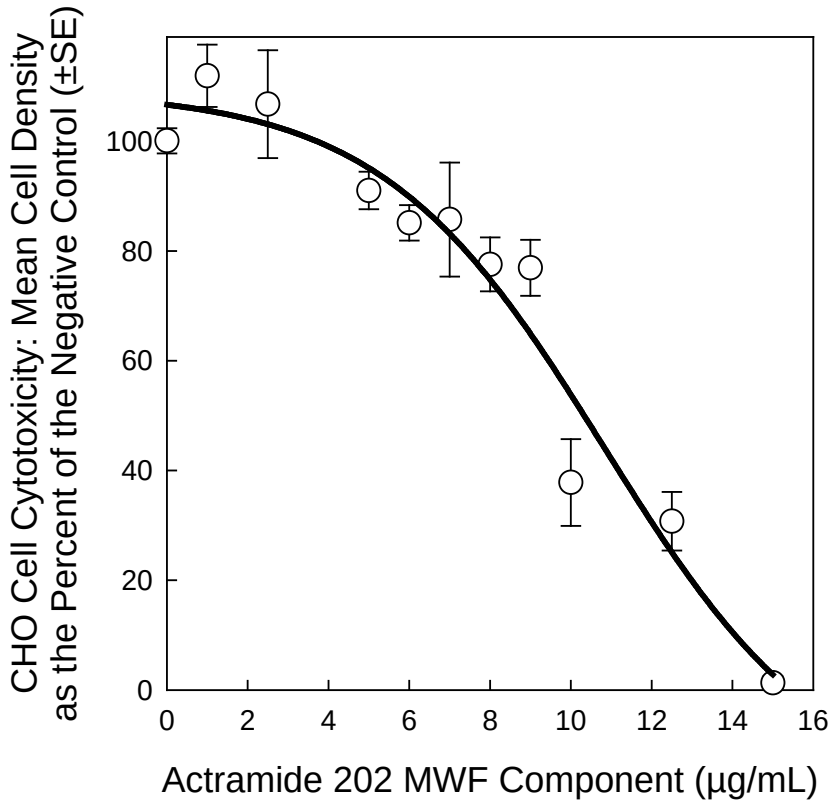

Figure 21. CHO cell chronic cytotoxicity concentration-response curve for Actramide 202.

Dover Mayfree 133

The summary data for the CHO cell cytotoxicity assay for the MWF component Dover Mayfree 133 is presented in Table 38 and the statistical analysis is presented in Table 39. Figure 22 presents the cytotoxicity concentration-response curve for Dover Mayfree 133.

Table 38. Summary of CHO cell cytotoxicity of Dover Mayfree 133.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 050608MPEW, 051308MPEW 052008MPEW Dover Mayfree 133 MWF CHO Cytotoxicity % NEGATIVE CONTROL SUMMARY																
0	1	10	15	20	25	30	33	35	36	37	38	39	40	45	50	µg/ml
0	0.2	2	3	4	5	6	6.6	7	7.2	7.4	7.6	7.8	8	9	10	µg/well
112.65	118.24	101.76			30.44	50.82	47.31		35.60	21.31	13.58	17.56	12.88		-6.04	
97.66	94.71	95.88			46.60	49.88	36.07	15.93	28.34	15.93	17.56	5.15	3.75		-6.65	
91.10	102.35	115.00			32.08	45.90	31.62	28.34	16.39	21.08	8.90	7.49	-4.22		-8.46	
89.23	86.18	105.00			14.29	48.95	37.00	25.29	22.25	17.80	11.01	-1.64	-11.24		-7.55	
93.44		94.56	83.99	76.13	18.74	40.52	51.52	26.23	14.75	15.93	-0.23	3.04	-7.96		-5.44	
92.97		84.89	93.05	84.29	38.41	44.73	34.89	27.87	27.87	16.39	14.75	3.75	7.26		-5.74	
107.73		93.66	105.14	100.30	45.43	57.61	47.54	40.28	32.79	27.63	13.58	14.29	14.75	-2.34	3.93	
114.99		122.36	121.75	116.01	42.62	48.71	40.05	41.69	37.00	26.70	21.55	23.42	22.01	7.03	2.42	
116.47		125.38	127.19	119.03	80.97	57.40	49.24	46.83					5.14	-3.63		
101.76		128.10	113.60	108.76	83.38	66.16	59.82	50.76					8.76	-7.55		
83.82		128.70	100.00	101.00	85.20	83.08	60.42	48.34					2.42	-7.55		
98.24		137.76	124.77	120.54	107.55	96.68	80.97	60.12					13.29	-5.14		
110.00					112.39	95.47	80.97	52.87					13.90	-4.83		
87.65					98.79	80.06	71.60	69.79					15.11	-5.44		
107.65					112.69	89.73	82.78	81.27					19.34	4.53		
93.53					109.37		73.11	60.42					11.18	1.51		
99.09																
88.82																
88.22																
101.21																
107.85																
108.46																
108.46																
96.98																
24	4	12	8	8	16	15	16	15	8	8	8	8	16	10	8	number
99.92	100.37	111.09	108.69	103.26	66.18	63.71	55.31	45.07	26.87	20.35	12.59	9.13	7.90	-2.34	-4.19	Average
1.95	6.81	4.98	5.57	5.75	8.98	5.12	4.47	4.73	2.97	1.67	2.28	3.00	2.37	1.60	1.65	SE
9.58	13.62	17.26	15.76	16.26	35.91	19.85	17.87	18.32	8.41	4.72	6.46	8.47	9.49	5.05	4.67	SD

Table 39. Statistical analysis (one way repeated measures ANOVA) of the CHO cell cytotoxicity data of Dover Mayfree 133.

Data source: Data 20 in MWF 040108.SNB

Treatment Name	N	Missing	Mean	Std Dev	SEM
DMF133 0µg/mL	24	0	99.916	9.577	1.955
DMF133 1µg/mL	4	0	100.368	13.622	6.811
DMF133 10µg/mL	12	0	111.088	17.257	4.982
DMF133 15µg/mL	8	0	108.686	15.759	5.572
DMF133 20µg/mL	8	0	103.259	16.257	5.748
DMF133 25µg/mL	16	0	66.184	35.905	8.976
DMF133 30µg/mL	15	0	63.713	19.845	5.124
DMF133 33µg/mL	16	0	55.307	17.874	4.468
DMF133 35µg/mL	15	0	45.068	18.315	4.729
DMF133 36µg/mL	8	0	26.874	8.408	2.973
DMF133 37µg/mL	8	0	20.345	4.723	1.670
DMF133 38µg/mL	8	0	12.588	6.461	2.284
DMF133 39µg/mL	8	0	9.133	8.472	2.995
DMF133 40µg/mL	16	0	7.898	9.488	2.372
DMF133 45µg/mL	10	0	-2.341	5.049	1.597

Source of Variation	DF	SS	MS	F	P
Between Subjects	23	24704.516	1074.109		
Between Treatments	14	237148.015	16939.144	105.339	<0.001
Residual	138	22191.312	160.807		
Total	175	302330.531	1727.603		

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant Toxic Response?
DMF133 0 µg/mL vs. DMF133 40 µg/mL	92.033	20.527	8.949E-044	0.004	Yes
DMF133 0 µg/mL vs. DMF133 45 µg/mL	92.149	17.692	2.641E-037	0.004	Yes
DMF133 0 µg/mL vs. DMF133 39 µg/mL	77.249	13.731	1.375E-027	0.004	Yes
DMF133 0 µg/mL vs. DMF133 38 µg/mL	73.795	13.117	5.000E-026	0.005	Yes
DMF133 0 µg/mL vs. DMF133 35 µg/mL	53.898	11.779	1.339E-022	0.005	Yes
DMF133 0 µg/mL vs. DMF133 37 µg/mL	66.037	11.738	1.704E-022	0.006	Yes
DMF133 0 µg/mL vs. DMF133 36 µg/mL	59.509	10.578	1.619E-019	0.006	Yes
DMF133 0 µg/mL vs. DMF133 33 µg/mL	44.623	9.953	6.346E-018	0.007	Yes
DMF133 0 µg/mL vs. DMF133 30 µg/mL	35.252	7.704	2.318E-012	0.009	Yes
DMF133 0 µg/mL vs. DMF133 25 µg/mL	33.746	7.527	6.089E-012	0.010	Yes
DMF133 0 µg/mL vs. DMF133 15 µg/mL	22.303	3.964	0.000118	0.013	No
DMF133 0 µg/mL vs. DMF133 10 µg/mL	17.495	3.566	0.000498	0.017	No
DMF133 0 µg/mL vs. DMF133 20 µg/mL	16.877	3.000	0.00321	0.025	No
DMF133 0 µg/mL vs. DMF133 1 µg/mL	16.526	2.266	0.0250	0.050	No

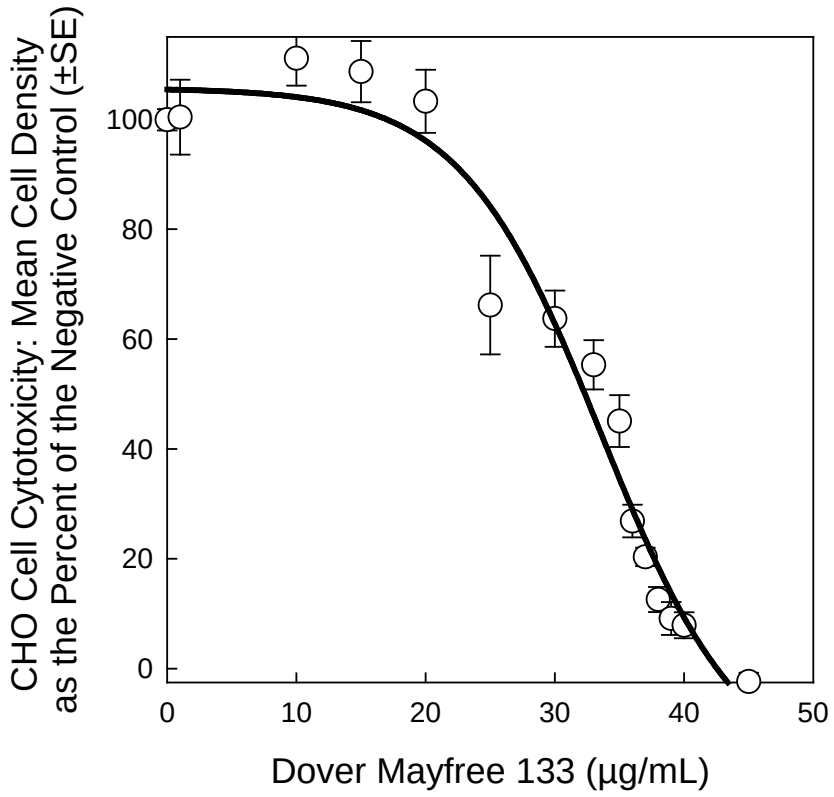

Figure 22. CHO cell chronic cytotoxicity concentration-response curve for Dover Mayfree 133.

Cover Sulperm HO

The summary data for the cytotoxicity assay for Dover Sulperm HO MWF component is presented in Table 40 with the statistical analysis presented in Table 41 and the concentration-response curve in Figure 23.

Table 40. Summary of CHO cell cytotoxicity of Dover Sulperm HO.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 050608MPEW, 051308MPEW, 052008MPEW PL1 Dover Sulperm ho MWF CHO Cytotoxicity % NEGATIVE CONTROL SUMMARY																					
0	1	10	50	100	150	200	250	300	325	350	375	400	425	450	460	470	480	500	550	600	µg/ml
0	0.2	2	10	20	30	40	50	60	65	70	75	80	85	90	92	94	96	100	110	120	µg/well
105.68	94.32	115.91	101.89	101.14	101.14	103.41	105.44	62.50	91.19	71.21	65.03	55.30	72.80	49.74	21.76	21.56	21.18	39.39	3.82	-8.29	
94.70	107.58	113.64	96.97	117.42	95.45	84.09	100.78	82.95	73.83	60.98	75.65	48.48	52.07	51.55	21.95	18.89	8.21	29.17	-1.72	-11.14	
88.64	110.23	112.50	109.85	113.26	98.11	88.64	104.40	48.11	90.41	41.67	76.68	18.18	62.44	54.66	24.24	23.66	21.95	21.21	1.15	-10.36	
95.08	102.27	124.62	119.32	116.29	105.30	87.50	95.34	50.38	87.31	50.76	68.13	28.41	61.40	59.84	24.24	31.11	30.34	24.24	0.38	-10.36	
96.59						99.48	104.92	84.97	106.22	63.99	93.26	58.29	81.61	57.25	25.76	42.18	35.69	15.84	0.19	-11.40	
93.94						93.78	98.70	92.75	111.40	72.02	69.69	63.73	57.77	33.16	28.05	34.54	41.22	9.92	-1.91	-14.77	
117.42						96.89	127.46	85.75	94.04	87.05	79.79	69.17	65.28	68.13	46.37	52.48	54.39	4.20	3.82	-7.25	
107.95						89.38	85.75	106.99	87.82	93.01	80.57	78.50	62.44	68.13	58.02	56.11	52.10	20.61	1.72	-8.03	
105.96						103.11		104.92		104.40		86.79	21.18	24.24				12.60			
81.87						95.08		115.80		79.02		51.30	37.98	35.50				21.56			
97.41						131.35		108.29		80.57		63.99	35.50	30.15				36.07			
92.75						115.80		83.94		61.92		53.37	37.79	34.92				27.86			
97.15								43.70		38.93		33.21	46.18	35.69							
92.75								56.11		50.95		42.37	61.26	51.72							
112.44								61.07		39.69		36.64	61.45	56.68							
120.21								53.82		44.47		47.33	50.76	58.02							
102.10								59.73		41.98		33.21									
90.08								74.81		53.44		18.32									
88.55								73.47		70.23		57.44									
90.08								51.72		62.21		69.08									
90.65																					
110.11																					
108.59																					
120.23																					
24.00	4.00	4.00	4.00	4.00	4.00	12.00	8.00	20.00	8.00	20.00	8.00	20.00	16.00	16.00	8.00	8.00	8.00	12.00	8.00	8.00	number
100.04	103.60	116.67	107.01	112.03	100.00	99.04	102.85	75.09	92.78	63.42	76.10	50.66	54.24	48.09	31.30	35.07	33.13	21.89	0.93	-10.20	Average
2.20	3.51	2.74	4.89	3.74	2.11	3.86	4.20	5.03	4.12	4.20	3.15	4.21	3.86	3.46	4.74	4.98	5.64	2.99	0.77	0.85	SE
10.80	7.02	5.49	9.77	7.47	4.23	13.37	11.89	22.49	11.65	18.79	8.91	18.82	15.44	13.83	13.42	14.07	15.95	10.37	2.18	2.40	SD

Table 41. Statistical analysis (one way repeated measures ANOVA) of the CHO cell cytotoxicity data of Dover Sulperm HO.

Data source: Data 21 in MWF 060908.SNB

Treatment Name	N	Missing	Mean	Std Dev	SEM
DSho 0 µg/mL	24	0	100.037	10.798	2.204
DSho 1 µg/mL	4	0	103.598	7.015	3.508
DSho 10 µg/mL	4	0	116.667	5.489	2.745
DSho 50 µg/mL	4	0	107.008	9.773	4.886
DSho 100 µg/mL	4	0	112.027	7.470	3.735
DSho 150 µg/mL	4	0	100.000	4.229	2.115
DSho 200 µg/mL	12	0	99.042	13.372	3.860
DSho 250 µg/mL	8	0	102.850	11.889	4.203
DSho 300 µg/mL	20	0	75.089	22.487	5.028
DSho 325 µg/mL	8	0	92.778	11.652	4.120
DSho 350 µg/mL	20	0	63.425	18.795	4.203
DSho 375 µg/mL	8	0	76.101	8.912	3.151
DSho 400 µg/mL	20	0	50.655	18.821	4.209
DSho 425 µg/mL	16	0	54.244	15.439	3.860
DSho 450 µg/mL	16	0	48.087	13.829	3.457
DSho 460 µg/mL	8	0	31.298	13.419	4.744
DSho 470 µg/mL	8	0	35.067	14.073	4.976
DSho 480 µg/mL	8	0	33.135	15.949	5.639
DSho 500 µg/mL	12	0	21.890	10.370	2.994
DSho 550 µg/mL	8	0	0.930	2.179	0.771

Source of Variation	DF	SS	MS	F	P
Between Subjects	23	11196.498	486.804		
Between Treatments	19	190063.458	10003.340	54.368	<0.001
Residual	173	31830.686	183.992		
Total	215	233981.675	1088.287		

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001). To isolate the group or groups that differ from the others use a multiple comparison procedure.

Power of performed test with alpha = 0.050:1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
DSho 0 µg/mL vs. DSho 550 µg/mL	101.606	17.331	1.165E-039	0.003	Yes
DSho 0 µg/mL vs. DSho 500 µg/mL	81.192	16.001	5.924E-036	0.003	Yes
DSho 0 µg/mL vs. DSho 460 µg/mL	71.238	12.151	5.847E-025	0.003	Yes
DSho 0 µg/mL vs. DSho 480 µg/mL	69.402	11.838	4.613E-024	0.003	Yes
DSho 0 µg/mL vs. DSho 470 µg/mL	67.469	11.508	4.030E-023	0.003	Yes
DSho 0 µg/mL vs. DSho 450 µg/mL	52.268	11.332	1.281E-022	0.004	Yes
DSho 0 µg/mL vs. DSho 400 µg/mL	47.911	11.169	3.712E-022	0.004	Yes
DSho 0 µg/mL vs. DSho 425 µg/mL	46.111	9.997	7.359E-019	0.004	Yes
DSho 0 µg/mL vs. DSho 350 µg/mL	35.141	8.192	5.462E-014	0.005	Yes
DSho 0 µg/mL vs. DSho 300 µg/mL	23.477	5.473	0.000000153	0.005	Yes
DSho 0 µg/mL vs. DSho 375 µg/mL	26.435	4.509	0.0000120	0.006	Yes
DSho 0 µg/mL vs. DSho 10 µg/mL	20.083	2.617	0.00966	0.006	No
DSho 0 µg/mL vs. DSho 100 µg/mL	15.443	2.012	0.0458	0.007	No
DSho 0 µg/mL vs. DSho 325 µg/mL	9.758	1.664	0.0979	0.009	No
DSho 0 µg/mL vs. DSho 50 µg/mL	10.424	1.358	0.176	0.010	No
DSho 0 µg/mL vs. DSho 1 µg/mL	7.015	0.914	0.362	0.013	No
DSho 0 µg/mL vs. DSho 200 µg/mL	4.039	0.796	0.427	0.017	No
DSho 0 µg/mL vs. DSho 150 µg/mL	3.416	0.445	0.657	0.025	No
DSho 0 µg/mL vs. DSho 250 µg/mL	0.314	0.0535	0.957	0.050	No

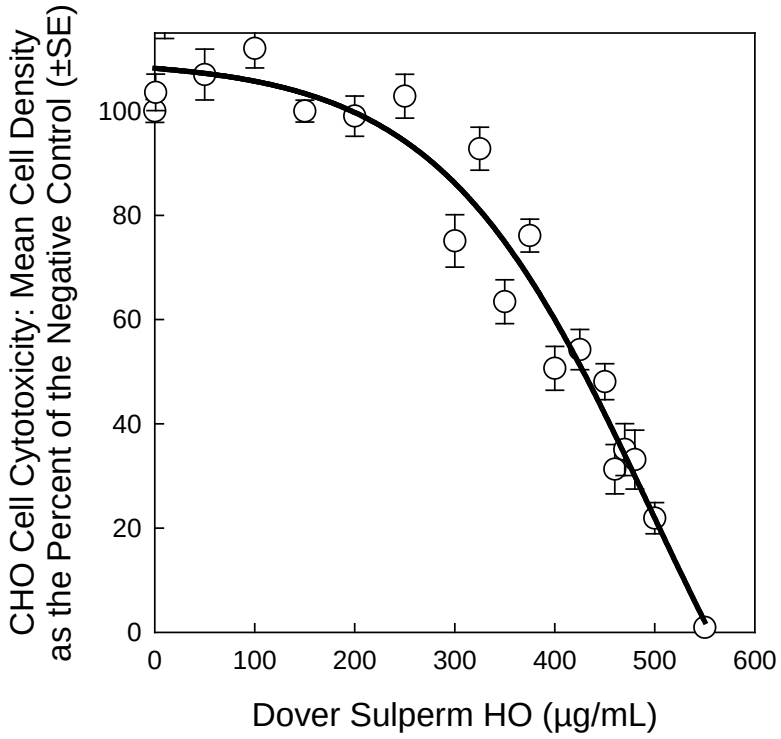

Figure 23. CHO cell chronic cytotoxicity concentration-response curve for Dover Sulperm HO.

Dover Maylube E112

The summary data for the CHO cell cytotoxicity assay for the MWF component Dover Maylube E112 is presented in Table 42 and the statistical analysis is presented in Table 43. Figure 24 presents the cytotoxicity concentration-response curve for Dover Maylube E112.

Table 42. Summary of CHO cell cytotoxicity of Dover Maylube E112.
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 050608MP, 081508MP 082508MPEW Dover Maylube E112 MWF CHO Cytotoxicity % NEGATIVE CONTROL													
0	10	50	100	150	175	200	225	250	275	300	350	450	µg/ml
0	2	10	20	30	35	40	45	50	55	60	70	90	µg/well
95.72	121.76	87.89	79.57	53.21	62.47	48.69	36.34	30.88	33.02	20.43	5.46	0.56	
109.98	105.88	77.67	69.83	51.78	44.42	39.19	42.52	37.05	34.20	23.99	3.56	0.28	
101.19	107.06	85.75	79.57	47.03	47.51	34.20	42.52	36.82	27.55	27.08	4.28	-3.92	
97.86		76.25	77.91	45.84	59.62	45.37	39.43	31.35	22.80	22.57	4.04	-1.12	
95.01		77.67	76.01	53.21	38.48	33.97	27.08	20.43	17.58	8.31	3.80	1.68	
99.52		99.52	64.37	43.94	36.58	38.95	22.57	19.00	10.45	14.25	3.09	-1.68	
116.47		79.10	69.12	56.77	43.47	30.88	38.72	30.40	17.81	14.25	8.55	19.33	
101.76		115.00		56.06	33.02	33.25	27.08	25.65	17.10	11.88	4.51	4.20	
83.82		96.76											
98.24		118.53											
110.00		103.24											
87.65													
107.65													
93.53													
98.04													
86.27													
95.80													
104.20													
100.28													
114.85													
100.84													
21.00	3.00	11.00	7.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	number
99.94	111.57	92.49	73.77	50.98	45.69	38.06	34.53	28.95	22.57	17.84	4.66	2.42	Average
1.88	5.11	4.59	2.26	1.70	3.73	2.22	2.76	2.40	2.97	2.33	0.61	2.56	SE
8.63	8.85	15.22	5.99	4.80	10.56	6.27	7.81	6.78	8.40	6.59	1.72	7.24	SD

Table 43. Statistical analysis (one way ANOVA) of the CHO cell cytotoxicity data of Dover Maylube E112.

Data source: Data 22 in MWF 060908.SNB

Group Name	N	Missing	Mean	Std Dev	SEM
MLE112 0 µg/mL	20	0	99.892	8.852	1.979
MLE112 10 µg/mL	3	0	111.569	8.850	5.109
MLE112 50 µg/mL	11	0	92.489	15.222	4.589
MLE112 100 µg/mL	7	0	73.770	5.985	2.262
MLE112 150 µg/mL	8	0	50.980	4.801	1.697
MLE112 175 µg/mL	8	0	45.695	10.563	3.735
MLE112 200 µg/mL	8	0	38.064	6.266	2.215
MLE112 225 µg/mL	8	0	34.531	7.807	2.760
MLE112 250 µg/mL	8	0	28.949	6.779	2.397
MLE112 275 µg/mL	8	0	22.565	8.398	2.969
MLE112 300 µg/mL	8	0	17.844	6.593	2.331
MLE112 350 µg/mL	8	0	4.662	1.722	0.609
MLE112 450 µg/mL	8	0	2.416	7.243	2.561

Source of Variation	DF	SS	MS	F	P
Between Groups	12	140284.084	11690.340	159.512	<0.001
Residual	100	7328.813	73.288		
Total	112	147612.897			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000
Multiple Comparisons versus Control Group (Holm-Sidak method): Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
MLE112 0 µg/mL vs. MLE112 400 µg/mL	97.476	27.218	4.921E-048	0.004	Yes
MLE112 0 µg/mL vs. MLE112 350 µg/mL	95.231	26.591	3.824E-047	0.005	Yes
MLE112 0 µg/mL vs. MLE112 300 µg/mL	82.048	22.910	1.406E-041	0.005	Yes
MLE112 0 µg/mL vs. MLE112 275 µg/mL	77.327	21.592	1.963E-039	0.006	Yes
MLE112 0 µg/mL vs. MLE112 250 µg/mL	70.943	19.810	2.149E-036	0.006	Yes
MLE112 0 µg/mL vs. MLE112 225 µg/mL	65.361	18.251	1.341E-033	0.007	Yes
MLE112 0 µg/mL vs. MLE112 200 µg/mL	61.828	17.264	9.225E-032	0.009	Yes
MLE112 0 µg/mL vs. MLE112 175 µg/mL	54.197	15.134	1.307E-027	0.010	Yes
MLE112 0 µg/mL vs. MLE112 150 µg/mL	48.912	13.658	1.357E-024	0.013	Yes
MLE112 0 µg/mL vs. MLE112 100 µg/mL	26.122	6.948	03.82 E-11	0.017	Yes
MLE112 0 µg/mL vs. MLE112 50 µg/mL	7.403	2.304	0.0233	0.025	Yes
MLE112 0 µg/mL vs. MLE112 10 µg/mL	11.676	2.203	0.0299	0.050	Yes

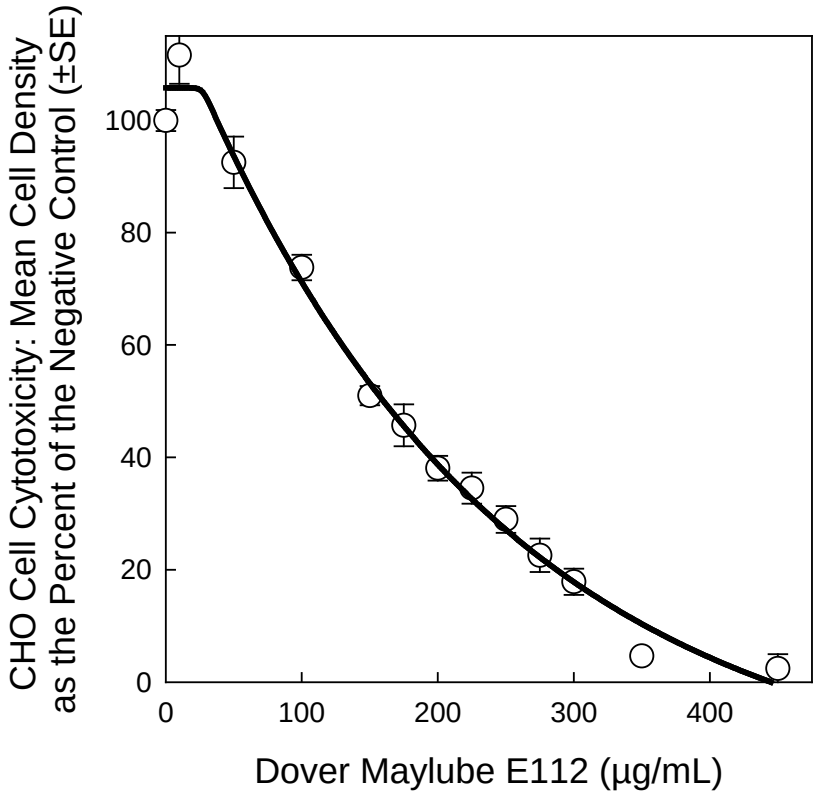

Figure 24. CHO cell chronic cytotoxicity concentration-response curve for Dover Maylube E112.

Dover Klorfree 100

The summary data for the CHO cell cytotoxicity assay for the MWF component Dover Klorfree 100 is presented in Table 44. As is indicated in the table, Dover Klorfree 100 does not exhibit cytotoxic activity (Figure 25). This is the only MWF or MWF component that we have assayed that demonstrates no cytotoxic response. We speculate that this lack of response is due to the insolubility of Dover Klorfree 100 in the DMSO/methanol stock solution and that this agent does not go into solution in the dilution series with the F12 cell medium (Figure 26). Thus, if Dover Klorfree cannot enter into the cell, its cytotoxicity activity cannot be addressed.

Table 44. **Summary of CHO cell cytotoxicity of Dover Klorfree 100.**
The cell density is a percentage of the concurrent negative control for each microplate.

Experiment 050608, 081508MPEW PL2 Dover Klorfree 100 MWF CHO Cytotoxicity % NEGATIVE CONTROL																				
0	1	10	50	100	150	200	300	350	400	450	500	550	600	650	700	750	800	900	µg/ml
105.68	134.47	129.55	139.77	146.59	145.08	132.20	112.50	114.02	101.14	117.24	75.00	127.59	149.57	143.97	127.59	145.69	156.90	131.03	
94.70	146.59	117.80	133.71	134.09	134.47	132.95	109.47	116.67	123.11	117.24	90.91	133.19	130.60	141.81	145.69	133.19	144.83	127.59	
88.64	145.08	131.44	127.27	153.79	135.98	143.18	131.06	147.35	113.64	121.12	106.82	128.45	143.53	134.05	142.24	125.43	130.60	128.88	
95.08	140.53	131.44	144.32	131.82	130.30	109.85	109.09	103.03	123.86	130.60	115.09	135.34	150.86	134.05	131.90	138.79	137.07	124.14	
96.59									121.55	100.86	134.91	127.59	141.38	132.76	120.26	131.03	130.60	122.84	
93.94									126.72	112.93	130.60	127.16	152.16	156.03	145.26	143.53	151.29	120.69	
117.42									113.36	120.69	120.69	158.62	165.09	164.22	166.38	144.83	151.72	163.79	
107.95									116.81	119.40	109.48	117.67	141.81	137.07	165.09	162.07	136.21	145.69	
119.40									106.90		134.91								
103.02									121.55		147.41								
86.21									136.21		123.28								
102.16									114.66										
84.48																			
100.00																			
96.55																			
106.47																			
16	4	4	4	4	4	4	4	4	12	8	11	8	8	8	8	8	8	8	number
99.89	141.67	127.56	136.27	141.57	136.46	129.55	115.53	120.27	118.29	117.51	117.19	131.95	146.88	143.00	143.05	140.57	142.40	133.08	Average
2.50	2.72	3.28	3.70	5.21	3.11	7.03	5.23	9.50	2.69	2.98	6.33	4.23	3.57	4.06	5.86	3.99	3.60	5.16	SE
10.01	5.45	6.56	7.41	10.42	6.23	14.05	10.47	19.00	9.31	8.42	21.01	11.96	10.10	11.48	16.58	11.30	10.19	14.60	SD

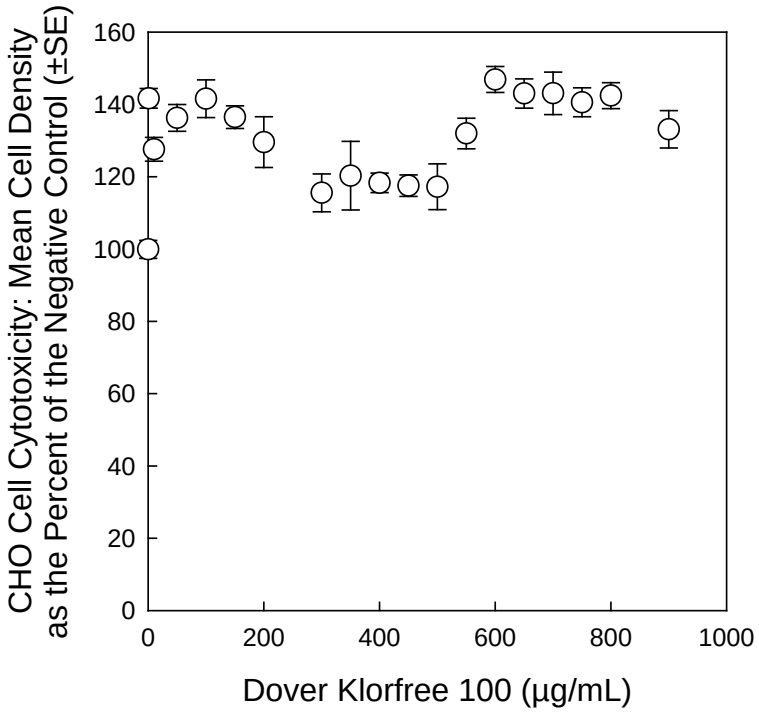

Figure 25. CHO cell chronic cytotoxicity concentration-response curve for Dover Klorfree 100.

Figure 26. Photograph of 10% Dover Klorfree 100 sample in a DMSO: methanol solution.

DISCUSSION

A comparison of the CHO cell cytotoxicity data for the 12 MWF agents plus the 10 MWF components analyzed thus far is presented in Table 45. The lowest toxic response value is the concentration of the agent that induced a significant reduction in the cell density as compared to the negative control. After regression analysis the coefficient of determination (R^2) was determined and the %C½ value for each concentration-response curve was calculated. The %C½ value (analogous to a LC_{50} value) is the concentration of the test agent that induced a cell density of 50% as compared to the concurrent negative control. A lower %C½ value denotes greater cytotoxicity.

For the MWFs presented in Table 45, the rank order based on their descending cytotoxicity (based on the %C½ value) is Castrol 6510 > Castrol Clearedge 6536 > Alusol AU39 > Cimperial 1070 > Vita Edge > Castrol 6519 > TrimSol > TrimE 20bnd > Eaton Hocut 763 > Hangsterfer's S506 > Syntilo 9904 > IRMCO Cutting Fluid Product A. For the MWF components the rank order based on their descending cytotoxicity (based on the %C½ value) is Actramide 202 > Alkaterg T-IV > Busan 77 > Dover Mayfree 133 > Dover Maylube E112 > AMP95 > UCON EMPL-48 > Dover Sulperm HO > UCON 50-HB-6. Dover Klorfree 100 was insoluble under conditions of the assay and thus could not be adequately analyzed.

Table 45. **Summary of the CHO cell cytotoxicity response by the MWF agents and components analyzed for this project.**

	MWF or Component	Lowest Toxic Response (µg/mL)	%C½ value (µg/mL)	R^2	ANOVA Test Statistic	Descending Rank Order
MWF	Castrol 6519	2.5	54.8	0.96	$F_{12, 183} = 98.3$; $P \leq 0.001$	6
	Cimperial 1070	2.5	41.2	0.99	$F_{15, 206} = 162.7$; $P \leq 0.001$	4
	Hangsterfer's S506	35.0	128.5	0.87	$F_{16, 234} = 162.7$; $P \leq 0.001$	10
	TrimE 20bnd	25.0	75.3	0.98	$F_{11, 212} = 104.6$; $P \leq 0.001$	8
	TrimSol	35.0	73.2	0.99	$F_{10, 163} = 107.9$; $P \leq 0.001$	7
	Castrol 6510	25.0	28.8	0.98	$F_{8, 151} = 37.6$; $P \leq 0.001$	1
	Eaton Hocut 763	50.0	105.9	0.99	$F_{8, 143} = 71.5$; $P \leq 0.001$	9
	Syntilo 9904	25.0	215.5	0.87	$F_{20, 202} = 61.2$; $P \leq 0.001$	11
	Vita Edge	10.0	46.1	0.95	$F_{10, 208} = 94.1$; $P \leq 0.001$	5
	Alusol AU39	5.0	32.0	0.98	$F_{13, 162} = 255.3$; $P \leq 0.001$	3
	Castrol Clearedge 6536	5.0	30.2	0.88	$F_{10, 173} = 130.2$; $P \leq 0.001$	2
	IRMCO Cutting Fluid Product A	85.0	405.2	0.89	$F_{20, 217} = 49.4$; $P \leq 0.001$	12
Component	Busan 77	5.0	25.1	0.97	$F_{8, 155} = 42.8$; $P \leq 0.001$	3
	UCON EMPL-48	125.0	261.3	0.96	$F_{14, 237} = 20.1$; $P \leq 0.001$	7
	AMP95	125.0	171.0	0.97	$F_{9, 147} = 21.3$; $P \leq 0.001$	6
	UCON 50-HB-6	200.0	429.2	0.89	$F_{14, 151} = 19.0$; $P \leq 0.001$	9
	Alkaterg T-IV	5.0	14.1	0.98	$F_{8, 98} = 170.9$; $P \leq 0.001$	2
	Actramide 202	8.0	10.1	0.95	$F_{10, 111} = 48.2$; $P \leq 0.001$	1
	Dover Mayfree 133	25.0	32.3	0.97	$F_{23, 14} = 105.4$; $P \leq 0.001$	4
	Dover Sulperm HO	350	428.6	0.96	$F_{23, 19} = 54.4$; $P \leq 0.001$	8
	Dover Maylube E112	50	164.9	0.99	$F_{12, 100} = 159.5$; $P \leq 0.001$	5
	Dover Klorfree 100	NA	NA	NA	NA	10

Table 46 presents the CHO cell cytotoxicity of U.S. EPA-regulated compounds. Figure 27 illustrates a comparison of the mammalian cell cytotoxicity of these agents with the MWFs from the present study. The comparison illustrated in Figure 27 indicates that the cytotoxicity of MWFs is similar to agents that are regulated by the U.S. EPA (black bars). In general there is an overall cytotoxicity rank order of MWFs (from most toxic to less toxic) of semi-synthetic > soluble oil > synthetic (Figure 28). This is an important discovery and is supported by an *in vivo* study of guinea pigs exposed to MWFs [15]. The CHO cell cytotoxicity data generated in this project are highly correlated with published *in vivo* pulmonary toxicology data (Table 47) [15].

Table 46. CHO cell cytotoxicity of selected U.S. EPA-regulated toxic agents.

Trade Name	Chemical Name	Type of Agent	%C½ Value (µg/mL)	Reference
2AAAF	2-Acetoxyacetylamino-fluorene	Arylamine carcinogen	3.16	[36]
Oxamyl	N,N-dimethyl-2-methyl-carbomyloxyimino-2-(methylthio)-acetamide	Carbamate insecticide	17.7	[35]
Atrazine	2-Chloro-4-ethylamine-6-isopropylamine-*s*-triazine	*s*-triazine herbicide	45.3	[35]
2,4-D	(2,4-dichlorophenoxy) acetic acid	Phenoxy herbicide	114	[35]

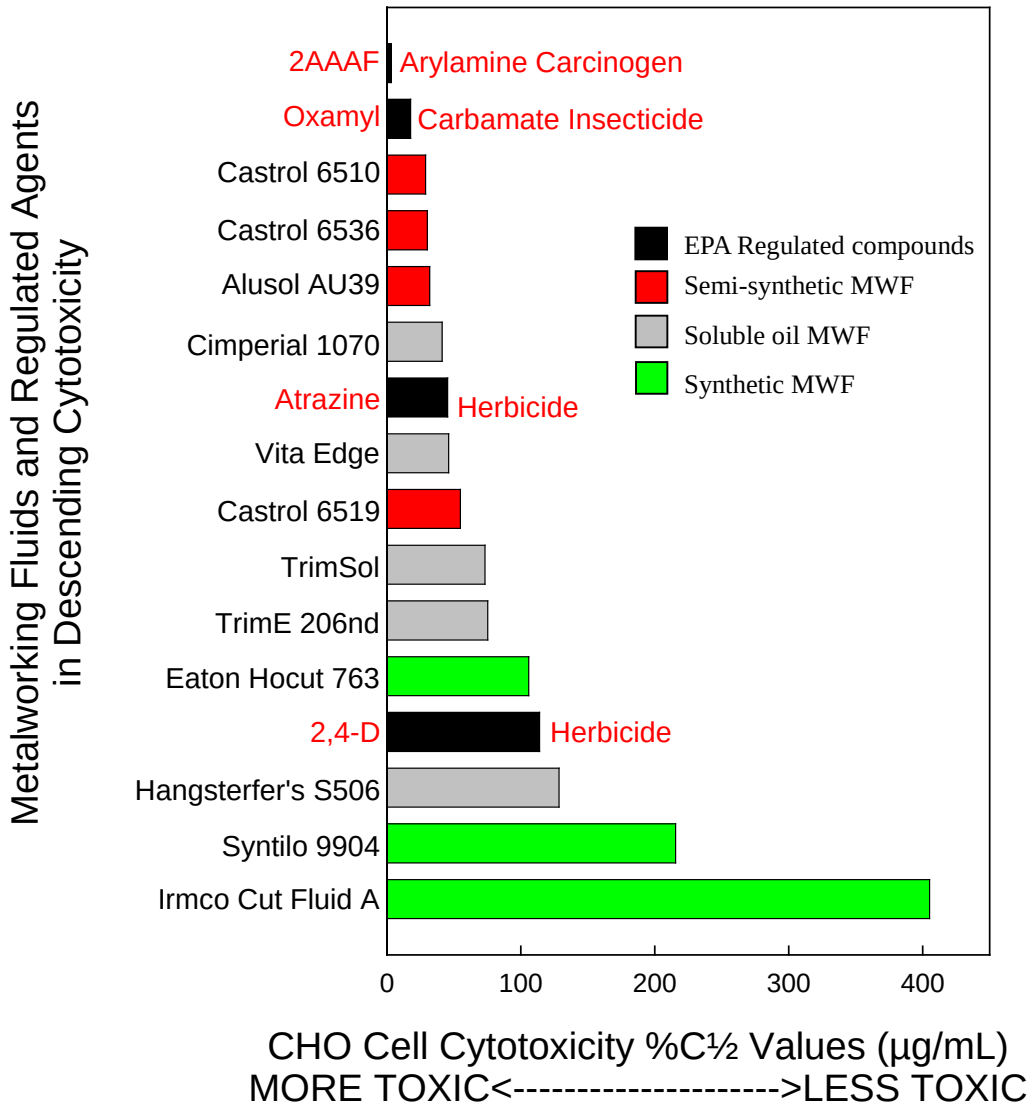

Figure 27. Comparison of the mammalian cell chronic cytotoxicity of the 12 metalworking fluids analyzed in this study. The red bars represent semi-synthetic MWFs, the gray bars represent soluble oil MWFs, and the green bars represent synthetic MWFs.

Table 47 compares the impact of various classes of MWFs and their average %C½ values from the CHO cell cytotoxicity assays with the release of LDH protein, overall protein and PMN as measures of pulmonary distress in guinea pigs exposed to MWFs [15]. The correlation responses are negative due to the fact that lower %C½ values indicate higher cytotoxicity (Table 45, Figure 27). Table 47 demonstrates that the *in vitro* CHO cell cytotoxicity assay is highly correlated to *in vivo* pulmonary toxicity measurements in animal models. This information suggests that the *in vitro* CHO cell cytotoxicity assay demonstrates a good level of confidence in predicting *in vivo* adverse health responses.

Table 47. Correlation analyses among *in vitro* and *in vivo* measurements of MWF toxicity.

MWF Type	Mean %C½ Value (µg/mL)	LDH BB (U/mL)	Protein (µg/mL)	PMN ($\times 10^6$)
Semi-synthetic	36.4	94.8	851.5	7.0
Soluble Oils	76.6	87.5	691.9	2.3
Synthetic	239.0	69.1	247.0	0.3
Pearson Product Moment Correlation Coefficient between %C½ values (present study) and *in vivo* toxicity measurements [15]		−0.99	−0.99	−0.85

To further analyze the cytotoxicity of the three classes of MWFs, we generated CHO cell cytotoxicity index values. The mean %C½ value for the MWFs was calculated for each MWF class (synthetic, soluble oil and semi-synthetic) based on mg/L concentrations. The reciprocal of each mean was calculated so that a larger value corresponded with increasing cytotoxicity. These cytotoxicity index values are useful in that we can quantitatively compare the relative CHO cell cytotoxicity amongst these three MWF classes. The data in Figure 28 clearly indicate that the soluble oil and semi-synthetic classes of MWFs were 3.2 × and 6.5 × more cytotoxic than the synthetic MWFs.

For the MWF components, the rank order based on their descending cytotoxicity (based on the %C½ value) is Actramide 202 > Alkaterg T-IV > Busan 77 > Dover Mayfree 133 > Dover Maylube E112 > AMP95 > UCON EMPL-48 > Dover Sulperm HO > UCON 50-HB-6. Dover Klorfree 100 was insoluble under conditions of the assay and thus could not be adequately analyzed. A comparison of the cytotoxicity of MWF components with known positive toxic agents and U.S. EPA-regulated compounds is presented in Figure 29. It is interesting to note that some of the components (Actramide 202, Alkaterg T-IV and Busan77) are more cytotoxic than the most cytotoxic MWF (Castrol 6510), and some components (Dover Sulperm HO and UCON 50-HB-6) are less cytotoxic than the least potent MWF (IRMCO Cutting Fluid Product A). This broad range of response indicates the possibility to employ specific MWF components to generate functional metalworking fluids that express reduced toxicity.

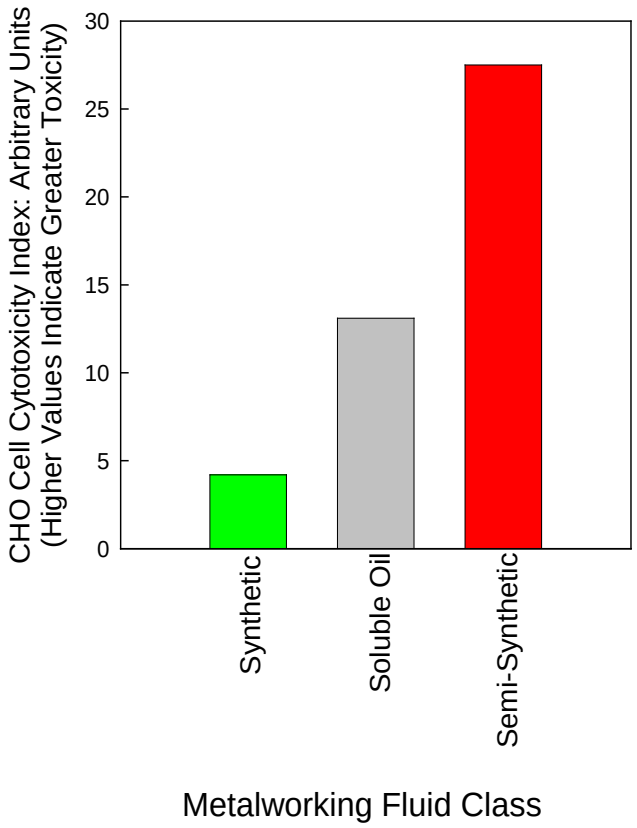

Figure 28. Comparison of the relative CHO cell cytotoxicity for the three metalworking fluids classes analyzed in this study.

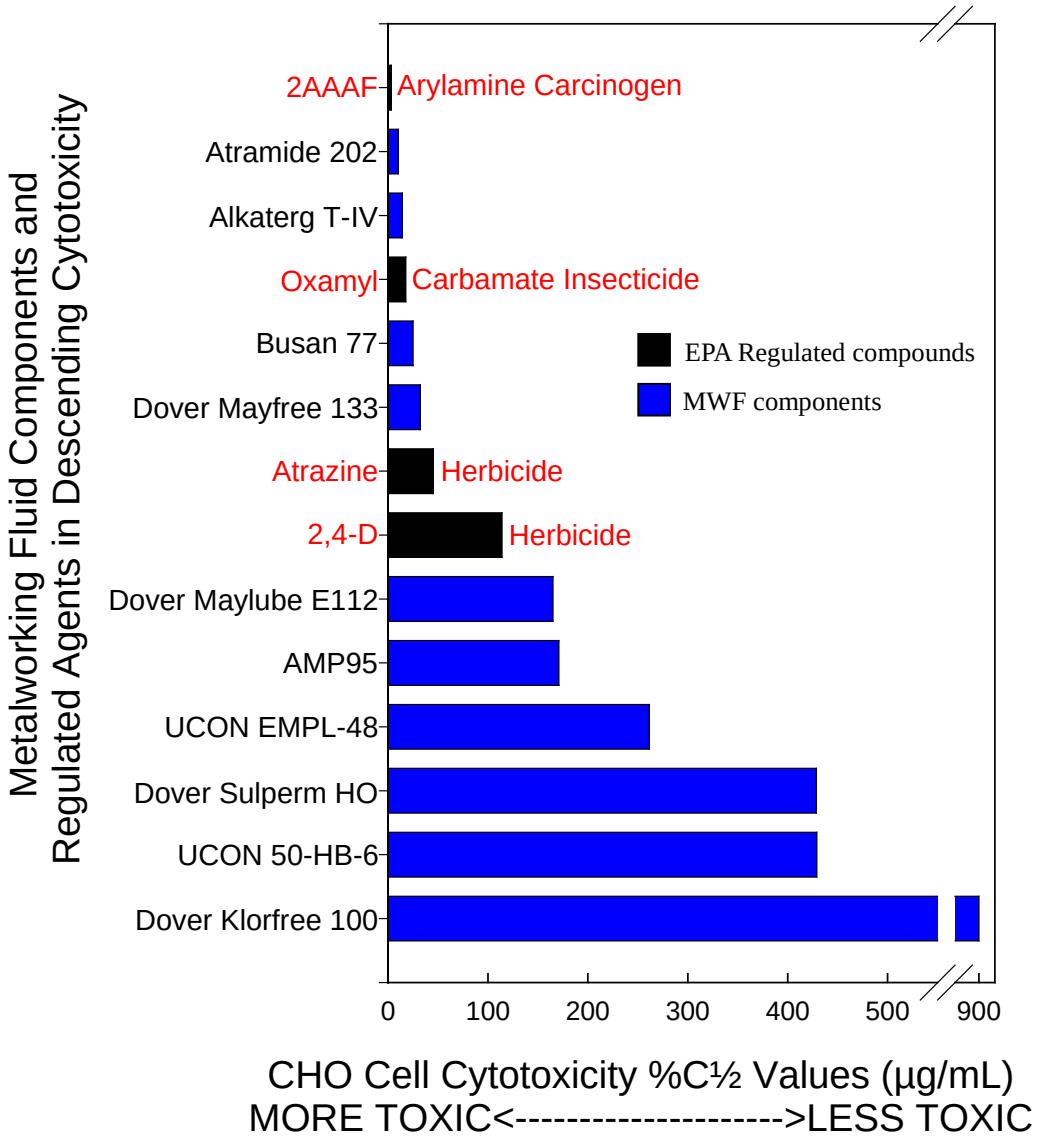

Figure 29. Comparison of the mammalian cell chronic cytotoxicity of the metalworking fluids components analyzed in this study.

CONCLUSIONS

From this research project the following may be concluded.

1. Twelve metal working fluids (MWFs) and ten metal working fluid components were evaluated for their chronic cytotoxicity using an *in vitro* CHO cell bioassay.
2. For both the MWFs and the MWF components, the range of cytotoxicity was within standard toxic agents regulated by the U.S. Environmental Protection Agency.
3. The MWF rank order, from most cytotoxic to least cytotoxic, was Castrol 6510 > Castrol Clearedge 6536 > Alusol AU39 > Cimperial 1070 > Vita Edge > Castrol 6519 > TrimSol > TrimE 20bnd > Eaton Hocut 763 > Hangsterfer's S506 > Syntilo 9904 > IRMCO Cutting Fluid Product A.
4. For the MWF classes, there is an overall descending cytotoxicity rank order of semi-synthetic > soluble oil > synthetic.
5. For these MWF classes, the CHO cell cytotoxicity assay was highly correlated to *in vivo* pulmonary toxicity measurements in animal models.
6. The MWF component rank order, from most cytotoxic to least cytotoxic, was Atramide 202 > Alkaterg T-IV > Busan 77 > Dover Mayfree 133 > Dover Maylube E112 > AMP95 > UCON EMPL-48 > Dover Sulperm HO > UCON 50-HB-6. Dover Klorfree 100 could not be analyzed.
7. For both the MWF and MWF components, a wide diversity of cytotoxicity is present. These data may be useful in selecting MWFs or MWF components that meet industry requirements and pose the lowest level of toxic hazard.

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